LD INTELLECTUAL PROPERTY ORGANIZA



Al

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 5: A61K 39/00, 39/395, C07K 7/10 C07K 15/06, 15/28, G01N 33/53

(11) International Publication Number:

WO 93/04695

A1

(43) International Publication Date:

18 March 1993 (18.03.93)

(21) International Application Number:

PCT/US92/07289

(22) International Filing Date:

27 August 1992 (27.08.92)

(30) Priority data:

750,913 817,912 28 August 1991 (28.08.91) US 6 January 1992 (06.01.92) US

JS |

(71) Applicants: THE WISTAR INSTITUTE [US/US]; 3601 Spruce Street, Philadelphia, PA 19104 (US). THE TRUSTEES OF THE UNIVERSITY OF PENNSYLVANIA [US/US]; The Center for Technology Transfer, 3700 Market Street, Suite 300, Philadelphia, PA 19104 (US).

(72) Inventors: WILLIAMS, William, V.; 25 Sycamore Road, Havertown, PA 19083 (US). WEINER, David, B.; 717 Beacon Lane, Merion, PA 19066 (US).

(74) Agents: CALDWELL, John, W. et al.; Woodcock Washburn Kurtz Mackiewicz & Norris, One Liberty Place - 46th Floor, Philadelphia, PA 19103 (US).

(81) Designated States: CA, JP, European patent (AT, CH, DE, FR, GB, IT).

Published

With international search report.

(54) Title: T CELL RECEPTOR-BASED THERAPY FOR RHEUMATOID ARTHRITIS

(57) Abstract

There is provided by this invention a novel method of treating rheumatoid arthritis in a mammal. The method comprises the steps of obtaining a sample of synovium from the mammal; identifying in said sample T cell receptor variable regions; and administering to said mammal an effective amount of antibodies to at least one of said T cell receptor variable regions or antigenic fragments thereof. The invention further provides a novel method of treating rheumatoid arthritis in a mammal comprising the steps of administering to said mammal an effective amount of antibodies to mammalian T cell receptor variable regions selected from the group consisting of $V\alpha17$, $V\alpha1$, $V\beta12$, $V\beta14$, $V\beta17$ and $V\beta7$ and antigenic fragments thereof. The invention further comprises a novel method for immunizing a mammal to prevent the occurrence of rheumatoid arthritis or to treat ongoing rheumatoid arthritis. The method comprises the steps of administering to said mammal mammalian T cell receptor variable regions selected from the group consisting of $V\alpha17$, $V\alpha1$, $V\beta12$, $V\beta14$, $V\beta17$, $V\beta14$, $V\beta17$, and antigenic fragments thereof. Kits comprising mammalian T cell receptor variable regions selected from the group consisting of $V\alpha17$, $V\alpha1$, $V\beta12$, $V\beta14$, $V\beta17$ and $V\beta7$ and antigenic fragments thereof or antibodies to said variable regions are also provided by the invention.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

		Fl	Finland	MN	Mongolia
AT	Austria	FR	France	MR	Mauritania
ΑÜ	Australia			MW	Malawi
BB	Barbados	GA	Gahon	NL	Netherlands
BE	Belgium	GB	United Kingdom	NO	Norway
BF	Burkina Faso	GN	Guinca		
BG	Bulgaria	GR	Greece	NZ	New Zealand
	-	HU	Hungary	PL	Poland
BJ	Benin	IE	Ireland	PT	Portugal
BR	Brazil	IT	Italy	RO	Romania
CA	Canada		· ·	RU	Russian Federation
CF	Central African Republic	JP	Japan	SD	Sudan
CG	Congo	KP	Democratic People's Republic		Sweden
CH	Switzerland		of Korea	SE	_
CI	Côte d'Ivoire	KR	Republic of Korea	SK	Slovak Republic
		LI	Licehtenstein	SN	Senegal
CM	Cameroon	LK	Sri Lanka	SU	Soviet Union
cs	Czechoslovakia		Laxembourg	TD	Chad
CZ	Cech Republic	เบ	_	TG	Togo
DE	Germany	MC	Монасо	UA	Ukraine
DK	Denmark	MG	Madagascar		United States of America
ES	Spain	MI	Mali	บร	Onneu states of America
	ala				

1

T CELL RECEPTOR-BASED THERAPY FOR RHEUMATOID ARTHRITIS

Cross Reference to Related Application

This application is a continuation-in-part of U.S. Serial No. 750,913 entitled "T Cell Receptor-Based Therapy for Rheumatoid Arthritis" filed in the U.S. Patent and Trademark Office on August 28, 1991 which is incorporated by reference herein.

Field of the Invention

This invention relates to the field of mammalian therapeutics. More particularly, methods of treating rheumatoid arthritis and methods for immunizing against rheumatoid arthritis are provided.

Government Rights

The work presented herein was supported in part by National Institute of Health grant 1R-29AI-28503-01. The United States Government has certain rights in the invention.

Background of the Invention

Rheumatoid arthritis (RA) is a systemic polyarthropathy characterized pathologically by proliferation of synovial fibroblast-like and macrophage-like cells and infiltration of the synovium with lymphocytes, predominately T cells of the helper (CD4+) phenotype (1,2). Such CD4+ T cells are typically activated by an antigenic peptide complexed with Class II MHC molecules (HLA-DR/DP/DQ). Immunogenetic analysis reveals that RA is associated with HLA-

WO 93/04695

DR4, and more specifically with glutamine/lysine residues at amino acids 70/71 of the HLA-DR β chain (3-8).

- 2 -

Current therapy for rheumatoid arthritis is either poorly efficacious or toxic. Many lines of evidence indicate that T cells are involved in the development of rheumatoid This includes the presence of lymphocytic joint disease. infiltrates composed primarily of CD4+ T cells in the synovium (2, 21-23) the linkage of RA to HLA-DR4 which comprises a (3-8), antigen receptors ligand for CD4+ T cell experimental models of arthritis and related autoimmune diseases which can be transferred by T cell lines (10, 13, 24-Studies in both animal models and human rheumatoid arthritis indicate that anti-T cell reagents can be of However, if these therapeutic efficacy (11, 25, 34-40). reagents are non-specific and delete too large a portion of the T cell repertoire, immunodeficiency (such as seen in acquired immune deficiency syndrome or AIDS) may result.

A better therapeutic alternative is to delete only those T cells involved in the autoimmune response. these comprise only a small portion of the total T cell repertoire, eliminating these T cells should not result in significant generalized immunosuppression.

Summary of the Invention

There is provided by this invention a novel method of treating rheumatoid arthritis in a mammal. The method comprises the steps of obtaining a sample of synovium from the mammal; identifying in said sample T cell receptor variable regions; and administering to said mammal an effective amount of antibodies to at least one of said T cell receptor variable regions or antigenic fragments thereof.

The invention further provides a novel method of treating rheumatoid arthritis in a mammal comprising the steps of administering to said mammal an effective amount of antibodies to mammalian T cell receptor variable regions selected from the group consisting of $V\alpha17$, $V\alpha1$, $V\beta12$, $V\beta14$, $V\beta$ 17 and $V\beta$ 7 and antigenic fragments thereof.

The invention further comprises a novel method for immunizing a mammal to prevent the occurrence of rheumatoid arthritis or to treat ongoing rheumatoid arthritis. The method comprises the steps of administering to said mammal mammalian T cell receptor variable regions selected from the group consisting of Val7, Val, V β 12, V β 14, V β 17, V β 7 and antigenic fragments thereof.

Kits useful in the methods of the present invention comprising mammalian T cell receptor variable regions selected from the group consisting of Va17, Va1, V β 12, V β 14, V β 17 and V β 7 and antigenic fragments thereof or antibodies to said variable regions are also provided by the invention.

Rheumatoid arthritis (RA) is characterized massive proliferation of synovial tissue, elevated expression of HLA DR antigens, accompanying infiltration of the tissue with CD4+ T lymphocytes, and a genetic linkage to the major histocompatility (MHC) antigen HLA-DR4. Since T cells are restricted by Class II MHC molecules such as DR4, this suggests a direct role for these CD4+ cells in pathogenesis. One strategy for the development of novel therapies in T cell to specifically delete the mediated autoimmunity is depends strategy cells. Such a autoreactive T understanding the molecular structure of autoreactive T cell To investigate the TCR usage in RA, receptors (TCR). oligonucleotide primers specific for each of the major TCR subfamilies - one set for the TCR alpha chains and one for the These were utilized to amplify TCR beta chains were used. cDNA derived from whole synovium or synovial tissue T cell lines in a family specific manner. Amplified cDNA was sequenced to determine the corresponding amino acid sequences. Detection of amplified DNA was facilitated by utilizing oligonucleotide probes derived from the constant regions of the TCRs. Synovial T cell lines were developed by stimulation with phytohemagglutinin followed by maintenance in IL-2. TCR repertoire present in these cell lines was quite heterogeneous, with an average of 15 alpha chains and 15.8 beta chains detected. When synovial tissue was analyzed, the predominant TCR subfamilies detected tended to be more restricted, with an average of 4.2 alpha chains and 9.7 beta chains detected. In some synovial tissue samples predominance of one subfamily was apparent. These results suggest that while a polyclonal population of T cells is present in RA synovium, the predominant patterns of TCR transcript expression may be somewhat more restricted. This suggests that TCR based therapy of RA is possible.

Brief Description of the Drawings

Figure 1. T cell receptor specific oligonucleotides and their relative location.

Figure 2. TCR transcripts in RA synovial T cell lines. Rheumatoid synovial T cell lines were developed by initial culture in PHA for 3-5 days, then maintained in IL-2 at 10 U/ml. Following 1-3 weeks of passage, the cells were frozen, and RNA later extracted for analysis of TCR expression as outlined in Materials and Methods. The sample designations are shown on the left, with the corresponding TCR alpha and beta family-specific primers used indicated above each lane.

Figure 3. TCR transcripts in RA synovium. RNA was extracted and cDNA synthesized form 10 rheumatoid synovial tissues obtained at the time of joint surgery. These were analyzed for TCR expression as noted above. The sample designations are shown on the left, with the corresponding TCR alpha and beta family-specific primers used indicated above each lane.

Figure 4. (A) Graphic representation of the frequency of occurrence of individual alpha chain variable regions in rheumatoid synovial tissue and T cell lines;

(B) Graphic representation of the frequency of occurrence of individual beta chain variable regions in rheumatoid synovial tissue and T cell lines.

Figure 5. T cell receptor PCR primers. The asterisk denotes antisense primer. $C\beta_1$ and $C\beta_2$ primers were used mixed together in equimolar concentrations.

Figure 6. T cell receptor β chain expression in ten rheumatoid synovia. The asterisk denotes > 2 standard errors from the mean.

Figure 7. T cell receptor α chain expression in ten rheumatoid synovia. The asterisk denotes > 2 standard errors from the mean.

Detailed Description of the Invention

In one aspect of the invention a method of treating rheumatoid arthritis in a mammal, such as a human, is provided. The method comprises obtaining a sample of synovium from the mammal; identifying in said sample T cell receptor variable regions; and administering to said mammal an effective amount of antibodies to at least one of said T cell receptor variable regions or antigenic fragments thereof.

Samples of synovium such as synovial tissue or fluid are obtained as is known to those in the art.

Molecular characterization of human T cell receptors has been greatly aided recently through the application of the polymerase chain reaction (PCR). (19) See also e.g. U.S. patent 4,386,202 issued to Mullis which patent is incorporated by reference as if fully set forth herein. By utilizing oligonucleotide primers specific for the different T cell specific family families, receptor variable region This technique can amplification is possible (14-16). conveniently be applied to the identification of T cell receptors of interest.

T cell receptors are generally Sequences of available in the literature and in computer-based sequence data bases such as "Genbank" and "EMBL". Thus, the sequence of the family-specific oligonucleotide primer of interest can be matched against these data bases utilizing a variety of computer software tools (For example, the University of Wisconsin package. (49)) with programs such as "Word Search The matched sequence are and Segments" or "Best Fit". retrieved from the data base and translated from nucleic acid to protein sequence. Alternatively, the T cell receptors of interest can be identified by in situ hybridization, Northern or Southern blot analysis of synovial fluid or tissue with immunohistochemistry by family-specific probes or immunofluorescence with antibodies to the various T cell receptor variable regions, where available.

An effective amount of antibodies to at least one of the T cell receptor variable regions is then administered

to the mammal. It should be noted that "antibodies to at least one of the T cell receptor variable regions" is meant to denote antibodies which recognize T cell receptor variable regions and portions or fragments thereof. An effective amount of antibodies is that amount which reduces the level of T cells bearing the corresponding receptor in the synovium or which results in clinical signs of improvement in the patient.

An antibody is said to be "capable of binding" a molecule if it is capable of specifically reacting with the molecule to thereby bind the molecule to the antibody. The term "epitope" is meant to refer to that portion of an antigen which can be recognized and bound by an antibody. An antigen may have one or more than one epitope. An "antigen" is a substance capable of inducing an animal to produce antibodies capable of binding to an epitope of that antigen. The specific reaction referred to above is meant to indicate that the antigen will immunoreact, in a highly selective manner, with its corresponding antibody and not with the multitude of other antibodies which may be evoked by other antigens.

The term "antibody" (Ab) or "monoclonal antibody" (Mab) as used herein is meant to include intact molecules as well as fragments thereof (such as, for example, Fab and $F(ab')_2$ fragments) which are capable of binding an antigen. Fab and $F(ab'_2)$ fragments lack the Fc fragment of intact antibody, clear more rapidly from the circulation, and may have less non-specific tissue binding.

The antibodies useful in the present invention may be prepared by any of a variety of methods. Antibodies useful in the present invention include antibodies to the T cell receptor variable region as well as antibodies to antigenic fragments thereof. Methods for the production of such antibodies are well known and described fully in the literature. (19) For example, cells expressing the peptide, synthetic peptides or an antigenic fragment thereof, can be administered to an animal in order to induce the production of sera containing polyclonal antibodies that are capable of binding the peptide. Peptides useful in the present invention

may range in size from about 25 to about 500 amino acids in length. In some embodiments of the present invention peptides may be from about 50 to about 300 amino acids in length. In still other embodiments of the present invention peptides may be from about 50 to about 200 amino acids in length. Generally, a peptide fragment is prepared and purified to render it substantially free of natural contaminants or a peptide fragment is synthesized, according to means known in the art. Either the purified fragment or the synthesized fragment or a combination of purified natural fragments and/or synthesized fragment may be introduced into an animal in order to produce polyclonal antisera of greater specific activity.

Monoclonal antibodies can be prepared using known hybridoma technology. In general, such procedures involve immunizing an animal with a peptide antigen, which includes the T cell receptor variable region and antigenic fragments thereof. The splenocytes of such animals are extracted and fused with a suitable myeloma cell line. Any suitable myeloma cell line may be employed in accordance with the present invention. After fusion, the resulting hybridoma cells are selectively maintained in a suitable medium and then cloned by limiting dilution. The hybridoma cells obtained through such a selection are then assayed to identify clones which secrete antibodies capable of binding the peptide antigen.

If the peptide source is impure, only some of the hybridoma cells will produce antibodies capable of binding to the peptide (other hybridoma cells will produce antibody capable of binding to the peptide contaminants). Thus, it may be necessary to screen among the hybridoma cells for those which are capable of secreting an antibody which is capable of binding to the peptide. Once such a hybridoma cell has been identified, it may be clonally propagated by means known in the art in order to produce the peptide-specific monoclonal antibody.

The sequence of many human T cell receptor variable regions are known and are available in data bases such as "Gen Bank" and "EMBL". Additional sequences of interest may be

WO 93/04695 PCT/US92/07289

- 9 -

determined by cloning and sequencing cDNA clones of T cell receptors isolated from synovial tissue or fluid (48).

In particular sequences of T cell receptor variable regions $V\beta14$, $V\beta17$, $V\alpha1$ and $V\alpha17$ are preferred. Preferred DNA sequences and corresponding amino acid sequences of these regions are set forth in Table 1. Table 1 sets forth preferred sequences of rheumatoid synovial T cell receptor α and β chain variable regions derived from human synovial tissue. Such sequences and portions of said sequences are useful for the development of antibodies useful in the present invention. It should be understood by those skilled in the art that, in some embodiments of the present invention nucleic acid analogs may be substituted for naturally occuring nucleic In preferred embodiments of the present invention nucleic acid sequences may range from about 75 to about 1500 nucleic acid bases in length based upon the portion of the T cell receptor variable region being coded and the size of a particular T cell receptor variable region. In other preferred embodiments nucleic acid sequences may range in length from about 150 to about 900 nucleic acid bases. In yet other embodiments of the present invention from about 150 to about 600 nucleic acids may code for a selected T cell receptor variable region or portion thereof.

Table I

I. Rheumatoid Synovial T Cell Receptor Beta Chain Nucleic Acid and Amino Acid Sequences

Patient 6, Clone β 14.1 (SEQ ID NO: Family Specific Primer V β 14 ÷

TAC AGT GTC TCT AGA Tyr Ser Val Ser Arg CCT GAA ST 1 Pro Glu Gly T 10 Gly Asp Val Pa AAG Thr Asp Lys GAT ACT GTG

Gln ACC AAC Thr Asn GAG TCC GCC AGC Glu Ser Ala Ser CTG Len 25 ATT Ile CIG Leu TTC TCC Arg 20 ပ္ပပ္ပ GAG Glu Lys AAG

Ser CCC AAC AGT Asn Pro Lys CAA AAA Gln AGT TCA Ser Ser GCC AGC A 40 \mathbf{TGT} Leu Cys CTC TAC ATG Met 35 \mathbf{TCT} Ser ACA

Asn GAG GAC CTG AAC Leu Asp Glu GTA Val GTT Val TCC Leu $\mathbf{T}\mathbf{T}\mathbf{G}$ AGG Arg ACC GGG TCGSer GGTGly Phe ACC

AAG Lys

Lys AAA

GAG GCA Glu Ala GAA Ser TCA Pro CCA GAG GCT GTG TTT Ala Val Phe $\mathbf{T}\mathbf{T}\mathbf{I}$ GTGGIC Val CCA CCC GAG Pro Pro Glu Phe Val 65

NO:3)	
I I	.
2 (SEQ	\mathbf{r} V β 1,
814.2	Prime
Clone	Specific P
int 6,	y Spe
Patient	Famil

GAG Glu
AGA Arg 15
TCT Ser
GTC Val
AGT
TAC
GGG Gly 10
GAA Glu
CCT
GTT Val
GAT
GGA Gly 5
AAG Lys
GAT Asp
ACT Thr
GTG Val

CAG		
AAC		
ACC	Thr	30
AGC	Ser	
ပ္သပ္သ	Ala	
TCC (Ser	
GAG	Glu	
TG	en	25
3 ATT C	Ile	
cTG	Leu	
TCC	Ser	
TTC	Phe	
	Arg	20
GAG	Glu	
AAG	Lys	•
AAG	Lys	•

걸길	四位
TTC	GTG
Phe	Val
GCT	AAG
Ala	Lys
GAA Glu 45	AAC Asn
ACT Thr	CTG Leu 60
666	gac
G1y	Asp
TGG	GAG
Trp	Glu
AGT	GTA
Ser	Val
AGC Ser 40	GTT Val
GCC	ACA Thr
TGT	CTC
Cys	Leu
CTC	AGA
Leu	Arg
TAC Tyr	ACC
ATG Met 35	GGC Gly
TCT	CAA
Ser	Gln
ACA	GGA
Thr	Gly

	GAG
9	GCA
	GAA
	TCA
	CCA
	GAG
ဂ	TTT
	GTG
	GCT
	GTC
	GAG
20	သသ
	CCA

Pro Pro Glu Val Ala Val Phe Glu Pro Ser Glu Ala Glu 65 3. Patient 5, Clone β 14.3/4/5/6 (BEQ ID NO:5) Family specific Primers $v\beta$ 14

GAG Glu GTC TCL ACT I Val Ser Arg G Ser CCT GAA GGG TAC AGT Pro Glu Gly Tyr 10 ACT GAT AAG GGA GAT GTT Thr Asp Lys Gly Asp Val GAG Glu

Gln ACC AAC Thr Asn CTG ATT CTG GAG TCC GCC AGC Leu Ile Leu Glu Ser Ala Ser 25 CGC TTC TCC C Arg Phe Ser L 20 GAG Glu AAG Lys AAG

ACA AGT TTG CTC CAG CGG ACC ACC Ser Leu Leu Gln Arg Thr Thr 45 GCC AGC AGT TTG CTC Ser 40 Ala Cys CTC TGT Leu TAC Tyr ATG Ser Met TCT ACA

TTT GGC CCA GGC ACC CGG CTG ACA GTG CTC GAG GAC Phe Gly Pro Gly Thr Arg Leu Thr Val Leu Glu Asp 55

Tyr

cAG Gln

Thr

GAT

50

ACG

TAT

GAA Glu 80 TIT GAG CCA TCA Phe Glu Pro Ser \mathtt{GTG} Val 75 TTC CCA CCC GAG ATC GCT Phe Pro Pro Glu Ile Ala 70 AAA AAC GTG Lys Asn Val CTG Leu

GCA GAG Ala Glu

NO: 7)	
A	
	Vβ14
8 B14.7	Primer
5, Clone	Specific P
Ĭ,	181
Patier	Family 8
•	

GAG Glu
AGA Arg 15
TCT Ser
GTC Val
AGT Ser
TAC Tyr
GGG G1Y .0
GAA Glu
CCT
GTT Val
GAT
GGA Gly 5
AAG Lys
GAT Asp
ACT Thr
GTG Val 1

rh C
CAG Gln
AAC Asn
ACC Thr 30
AGC Ser
GCC Ala
TCC Ser
GAG Glu
CTG Leu 25
ATT Ile
CTG Leu
TCC
TTC Phe
CGC Arg 20
GAG Glu
AAG AAG Lys Lys
AAG Lys

TAC Tyr
CAG Gln
GAG Glu
GGC G1Y 45
AGG Arg
gac Asp
CTG Leu
AGC
AGC Ser 40
GCC Ala
TGT Cys
CTC
TAC Tyr
ATG Met 35
TCT Ser
ACA

GTG	Val	
AAC	Asn	
AAA	Lys	
CTG	Len	
GAC	Asp	9
GAG	Glu	
ACA	Thr	
GTC	Val	
ACG	Thr	
CIC	Len	5
AGG	Arg	
ACC	Thr	
၁၅၅	Gly	ı
SCG	Pro	
	Gly	R C
TIC	Phe	

GAA GCA GAG Glu Ala Glu TTC CCA CCC GAG GTC GCT GTG TTT GAG CCA TCA Phe Pro Pro Glu Val Ala Val Phe Glu Pro Ser 65

(6:0N QI	
(SEQ I	νβ14
Clone \$14.8	Specific Primer
ient 5, (Family Spec
i. Pat	Fam

GAG Glu	
AGA Arg	വ
TCT	_
GTC '	
AGT	
TAC	
A GGG 7	0.
GAA Glu	_
CCT	
GTT Val	,
GAT	
GGA Gly	ខ
AAG Lys	
GAT	
ACT	
GTG Val	-

ACC AAC (Thr Asn (30 ATT CTG GAG TCC GCC AGC Ile Leu Glu Ser Ala Ser G GAG CGC TTC TCC CTG A Glu Arg Phe Ser Leu I. AAG AAG Lys

ACA GAT Thr Asp TTA ACC TCC GTC A GCC AGC AGT Ala Ser Ser TAC CTC TGT Tyr Leu Cys ATG Met 35 TCT Ser ACA

CTC GAG GAC CTG AAA Leu Glu Asp Leu Lys 60 GTG Val CTG ACA Leu Thr CGG ACC GGC CCA GGC Gly Pro Gly TAT TTT (Tyr Phe (CAG Gln

GAA Glu CCA TCA (Pro Ser (GAG Glu 75 CCA CCC GAG GTC GCT GTG TTT Pro Pro Glu Val Ala Val Phe 70 GTG TTC (Val Phe)

TCA GAA GCA GAG Ser Glu Ala Glu

1	
ID NO:11)	
H	
(8EQ	Vβ17
β 17.1	rimer
t 6, Clone	cific
9	Spe
Patient	Family

AAG Lys	ACA Thr	GGG G1y	CTG	CCA Pro 80
GAG Glu 5	CCG	ACC Thr	GTG Val	GAG Glu
cgg gAG Arg Glu 15	AAC Asn 30	CTA	TCA Ser	TTT Phe
TCT Ser	AAG Lys	GGG G1y 45	CCC	GT G Val
GTC Val	CAA Gln	CAA Gln	CGG Arg 60	GCT Ala
AGC Ser	GCC	GGA Gly	ACC Thr	GTC Val 75
GGG TAC Gly Tyr 10	TCG Ser	666 G1y	666 G1y	GAG Glu
666 61y 1	ACA Thr 25	ATT Ile	GCC Ala	CCC
GAA Glu	GTG Val	AGT Ser 40	66c 61y	CCA Pro
GCT Ala	ACT Thr	AGT Ser	TTC Phe 55	TTC
ATA Ile	CTC	GCC	TAC Tyr	GTG Val 70
gat Asp 5	CCT	TGT	CAG Gln	AAC Asn
GGA Gly	TTT Phe 20	CTC Leu	ATT Ile	AAA Lys
AAA Lys	TCC Ser	TAT TYr 35	AAC Asn	CIG
CAG Gln	GAA Glu	TTC Phe	AAA Lys 50	GAC Asp
TTT Phe 1	AAG Lys	GCT	GCC	GAG Glu 65

$\nabla \beta 17$
Family Specific Primer
Fami

GAG Glu	ACC Thr	CAG Gln	AAC Asn	
AAA Lys 5	CAG Gln	GAG Glu	AAA Lys	GAG Glu
CGA AAA Arg Lys 15	AAC Asn 30	AAT Asn	CTG	GCA Ala
TCT Ser	CCC Pro	TAC TYr 45	GAC Asp	GAA Glu
GTC Val	AGC Ser	ACC Thr	GAG Glu 60	TCA
AAA	CCC Pro	GGA Gly	CTA	CCA Pro 75
TAC TYr 0	TCG Ser	GGG G1 Y	GTG Val	GAG
GGG TAC AGIN TAT I	GAG Glu 25	TTG	ACC Thr	TTT Phe
GAA Glu	CTG Leu	AGT Ser 40	CTC	GTG Val
GCT Ala	ATC Ile	AGC Ser	CGG Arg 55	GCT
ATA Ile	CTG Leu	GCC Ala	ACA Thr	GIC Val 70
GAT Asp 5	CCC	TGT Cys	GGG G1y	GAG Glu
GGA	TTC Phe 20	TTC	CCA	CCC
AAA Lys	AAT Asn	TAC TYr 35	GGG	CCA Pro
CAG	AGG Arg	CTG Leu	TTC Phe 50	TTC Phe
TTT Phe 1	AAG Lys	TCT	TTC	GTG Val

ID NO: 33) (SEQ VB17 Patient 6, Clone \$17.3 Family Specific Primer

GAG Glu
AAA Lys L5
CGA AAA Arg Lys 15
TCT
GTC Val
TAC AAA Tyr Lys L0
TAC Tyr LO
GGG G1у.
GAA Glu
GCT
ATA Ile
GAT Asp 5
GGA Gly
AAA Lys
CAG Gln
rrr Phe 1

ACC Thr TCG CCC AGC CCC AAC CAG Ser Pro Ser Pro Asn Gln GAG Glu 25 Leu ATC (Ile Phe Pro Leu Asn AGG Lys Arg AAG

TAT Tyr TCC GCA Ala 45 Arg TCT TTC Pro ဌ္ဌ AGT GCC AGC A Cys TGT TTC Phe TAC TYF 35 Leu CTG TCT Ser

TTA

GGG AAC AGG

 \mathbf{TCG}

GAA GCA G GAC CTG GAG TCA GAG CCA 9 Glu Pro 9 75 GTA Val GTT Val ACC Phe $\mathbf{T}\mathbf{T}\mathbf{I}$ Asn Arg Leu 55 GAG GTC GCT GTG Glu Val Ala Val Gly Pro Ser ပ္ပပ္ပ GGT CCA TTC (Phe TTC ACC Thr 50 GTG Val AAC Asn 65 TAC

II. Rheumatoid Synovial T Cell Receptor Alpha Chains Nucleic Acid and Amino Acid Sequences

Tyr Leu Phe Trp TTC TGG TAT AGG TGC AAC TAT TCC TAT GGG GCA ACA CCT Arg Cys Asn Tyr Ser Tyr Gly Ala Thr Pro Patient 1, Clone α 1.1/2 (SEQ ID NO:15) Family Specific Primer Val Tyr Ser 5 Arg Cys Asn Leu CTG

Ser TLL Tyr Phe TAC Ser Pro Gly Gln Gly Leu Gln Leu Pro Leu Lys CTG AAG ပ္ပပ္ပ CAG CTG CIC ညည CAA 299 TCC CAG Gln Val

AAG TTT GAA GCT GAG TTT295 GGC ATT AAA CAA GTTCIG GAC ACT Gly

Phe Lys Gla Ala Glu Phe (lle Lys Gly Gln Gly Thr Leu Val Asp

Ser AGT TGG Trp His CAT GTG Val \mathbf{ICI} Ser Pro CCC AAA Phe Asn Leu Arg Lys AGG CIG TTC AAT TCC Ser TCT Ser CAA Gln Ser AGG AGT

Arg

AGC Ser TAC $\mathbf{T}\mathbf{y}\mathbf{r}$ GGA Gly Ser GCT GAT TCA Ala Asp GGT Val Gly GTG GCT Cys Ala \mathtt{TGT} Phe TTC TAC Glu Tyr GAG Ala GCT Ala GCT Asp 65

Ile ATC Asp 95 GAT Pro CCA TCT Ser ACT ATG CTT CTA GTC Thr Met Leu Leu Val 999 Gly CTC ACC TIT GGG AAG Gly Lys Phe Leu ACC

TCC Ser AAA $\frac{\text{Lys}}{110}$ TCT Gln Leu Arg Asp Ser AGA GAC CTG CAG 105 TAC Tyr Val GCC GTG Asp Pro Ala CCT GAC CCT Pro Asn AAC

AAG Lys GAC Asp

AGT GAC AAG Ser Asp Lys 110

CCT GAC CCT GCC GTG TAC CAG CTG AGA GAC TCT AAA TCC Pro Asp Pro Ala Val Tyr Gln Leu Arg Asp Ser 100

ID NO:17)	
(SEQ	V α1
2, Clone a1.3	Family Specific Primer
10. 1	

тат Туг	TCA Ser	AAG Lys	AGT Ser	ATG Met 80	AAC Asn
TGG Trp 5	TTT Phe	TTT Phe	TGG Trp	GAC Asp	CAG Gln 95
TTC TGG Phe Trp 15	TAC Tyr 30	GAA Glu	CAT His	AAT Asn	ATC Ile
CTC	AAG Lys	GCT Ala 45	GTG Val	CAC His	AAT Asn
TAT Tyr	CTG	GAG Glu	TCT Ser 60	ACC Thr	CCA Pro
CCT	CTC Leu	TTT Phe	CCC	CCC Pro 75	AAA Lys
ACA Thr	CTG	66c 61y	AAA Lys	GGT	GTA Val 90
GCA ACA Ala Thr 10	CAG Gln 25	AAA Lys	AGG Arg	GTG Val	ACA Thr
GGG G1y	CTC	ATT Ile 40	CIG	GCT Ala	CTG
TAT Tyr	66C 61y	GGC G1у	AAT Asn 55	TGT Cys	AGA Arg
TCC	CAA Gln	CAA Gln	TTC Phe	TTC Phe 70	ACC Thr
TAT Tyr 5	GGC Gly	GTT Val	TCC	TAC Tyr	GGG G1Y 85
AAC Asn	CCC Pro 20	CTG Leu	TCT Ser	GAG Glu	GCA Ala
TGC Cys	TCC	ACT Thr 35	CAA Gln	GCT	GGA Gly
AGG Arg	CAG Gln	gac Asp	AGT Ser 50	GCT Ala	TTT Phe
CTG Leu 1	GTC	GGA Gly	AGG	GAT Asp 65	CGC Arg

Patient 2, Clone $\alpha 1.4$ (SEQ ID NO: 19) Family Specific Primer $V\alpha 1$ 11.

CTC TTC TGG Glu Leu Phe Trp GAA CCL Gly Ser Pro GGG AGT \mathbf{TCT} Ser TAT TAT TCC 1 Tyr Ser 1 5 AAC Asn TGC Cys AGG Leu

CAC ATC His Ile Arg AGA CAG TTA CTC TTG Gln Leu Leu Leu CTC Leu CGC Gln Arg CAA AGA Arg Ser TAC Gln CAG GTC

ACA GAG GGC Gly Lys 45 AAA Asn GAC CTT AAC Leu Ala Asp GCT ACT Thr TTC Phe AAA GGC ! Lys Gly] ATC AGC GAG Glu AGA

Met ggg TCA Ser GAC Asp GAA Glu GAG Glu Gln CAA TTT GCT Phe Ala AAA CCA : Lys Pro 1 AAG CTG TTC CAC (Phe His 1 Ser

Glu GAG Leu CTT Leu Leu AGC TTA (Ser Leu 1 ACA Thr CAG GCA Gln Ala CTG Leu CTA GCG (Leu Ala GCT Ala TGT Cys TAC Tyr 65

Pro Ala ATC CAG AAC CCT GAC CCT Ile Gln Asn Pro Asp Pro CCA AAT AAA Lys GTTLeu Val CTA Val GTG AGG Arg CCC GAA Glu

gcc

Glu

Lys GAC Asp AGT Ser $\mathbf{T}^{\mathbf{CC}}$ Ser 105 TCT AAA Ser Lys AGA GAC Arg Asp Leu CAG Gln Tyr GTG

12. Patient 2, Clone a17.1 (SEQ ID NO: 25) Family Specific Primer Va17

Ser GTC AGC Val TAC ACA Tyr Thr TTG TCA CTG GAT TTA GAG TCT CTC AGC TGC AGT Leu Ser Leu Asp Leu Glu Ser Leu Ser Cys Ser 1

Leu Arg Gly Leu Phe Trp Tyr Arg Gln Asp Pro Gly Lys AAA 999 CAA GAT AGG TAT TTC GGG TTA AGA

Glu AAA Glu Lys Glu Lys AAG GAA TCA GCT GGG GAA Ser Ala Gly Glu TTC ACC CTG TAT Phe Thr Leu Tyr Phe Leu CIC

CAC Phe Leu His TTT Lys Lys Glu Ser 55 GAA AGC ACA TTA ACA AAG AAG Leu Thr Thr ညည Ala Lys Leu AGG

GTG AGG LIGT GCT GTG . Cys Ala Val A 75 CTC TGT Leu Tyr GCC ACT TAT Ala Thr Ser 70 TCA GAC Asp GAA Glu CCI Pro AAA Lys Pro

GAT GGC CAG AAG CTG CTC TTT GCA AGG GGA ACC ATG TTA AAG Asp Gly Gln Lys Leu Leu Phe Ala Arg Gly Thr Met Leu Lys 85

TCA

CTG AGA CAG Tyr GTG TAC Val GAC CCT GCC (Asp Pro Ala GÀC Pro CCL CAG AAC Gln Asn ATC Ile AAT Asn Leu

Patient 3, Clone α17.2 (SEQ ID NO:23) Family Specific Primer Vα17

15 Ser GIC AGC Val TAC ACA (TYR Thr V C TTG TCA CTG GAT TTA GAG TCT CTC AGC TGC AGT Leu Ser Leu Asp Leu Glu Ser Leu Ser Cys Ser 1

Gly Pro GGC CAA GAT CCT GGG AAA Gly Pro Asp Gln AGG Arg TAT Tyr CTG TTC TGG Leu Phe Trp 20 666 G1y TTA AGA Leu Arg Gly

Lys AAA GAG Glu Lys AAG GAA Glu GAA Glu GGG G1y GCT Ala Ser TCA TAT CTG Leu ACC TTC Leu Phe GAA Glu

ATC Ile CAC His CTG Leu Phe $\mathbf{T}\mathbf{T}\mathbf{T}$ AGC Ser GAA Glu Lys AAG AAG Lys 55 ACA Thr TTA Leu ACA Thr GCC Lys 50 Leu AGG

Arg GTG Val GCT Ala Cys 75 \mathtt{TGT} CTC Leu TAT Thr ACT GCC TCA Ser GAC Asp GAA Glu CCT Pro Lys AAA Pro GCC

AAG TTA Leu ACC Thr $_{
m G1y}$ GGA AGG Arg GCA Phe Ala $_{
m LLL}$ Leu CTC Leu CTG 85 Lys AAG CAG Gln Gly Asp GAT Ser

GAC Arg Leu CTGCAG Gln Tyr TAC GCC GTG Ala Val GTG Pro CCI CCT GAC (Pro Asp] Asn AAC Gln ATC Ile Asn Leu Asp GAT

14. Patient 4, Clone α17.3 (8EQ ID NO: 21) Family Specific Primer Vα17

TTG TCA CTG GAT TTA GAG TCT CTC AGC TGC AGT TAC ACA GTC AGC Leu Ser Leu Asp Leu Glu Ser Leu Ser Cys Ser Tyr Thr Val Ser 1 5 O

CCT Gly Lys AAA CAA GAT CCT GGG Gln Asp Pro Gly AGG Arg TAT CTG TTC TGG Leu Phe Trp Gly **88** TTA AGA Leu Arg

AAA Glu AAG Lys GAA Glu GAA Glu GGG Ala GCT TCA Ser TAT Tyr Leu CTG ACC Thr Phe Len

CAC His Phe Leu AGC GAA Glu Lys AAG Lys AAG ACA Thr TTA Leu ACA Thr GCC Lys Leu Arg

Ala GCT TGT Cys 75 CIC Leu TAT ACT Thr GCC Ser TCA GAA GAC CCT Pro Pro Lys Ala

Lys AAG Leu ATG Met ACC Gly Arg GCA AGG Ala Phe Leu crcLeu 85 CAG AAG Galn Lys 1 G1yပ္ပင္ပင Asp Ser 80 CAG CTG AGA Gln Leu Arg Tyr TAC GTG Pro Asp Pro Ala CCT GCC GAC CCT Ile Gln Asn AAC ATC CAG AAT Asn Leu CIL GAT

Patient 4, Clone α17.4 (SEQ ID NO: 27) Family Specific Primer Vα17

GIC Thr Val ACA TAC Ser Tyr TCT CTC AGC TGC AGT Ser Leu Ser Cys Ser 10 CTG GAT TTA GAG TCT Leu Asp Leu Glu Ser C TTG TCA Leu Ser

AAA Gly CAA GAT CCT G Gln Asp Pro G TAT AGG (Tyr Arg (TGG CTG TTC ! Leu Phe ! AGA Arg Leu GGT

AAA Lys Glu Lys AAG GAA Gla GAA Glu GGG Gly Ala GCT Ser TCA Leu GCC A TTC Len TTC

ATC Ile CAC His Leu CTGPhe TTT AGC GAA Glu Lys AAG AAG Lys 55 ACA Thr TTA Leu Thr GCC Lys Leu Arg

Arg GTG Val GCT Cys \mathbf{TGT} cIcLeu TAT ACT GCC Ala TCA Ser GAC Asp Glu Pro Lys AAA Pro

Val Leu ATG ACC Gly AGG Arg GCA Ala TTT Phe Leu CIC Leu 85 CTGLys Gln Gly Asp GAT Ser

CAG CTG AGA Gln Leu Arg Tyr TAC GTG Ala Pro CCTCCT GAC (Pro Asp 1 Asn AAC Gln 100 ATC Ile AAT Asn Leu

16. Patient 7, Clone α17.5 (SEQ ID NO: 29) Family Specific Primer Vα17

$^{ m LCG}$	Trp	15
ACC	Thr	
AAC	Asn	
TTT	Phe	
ATA	Ile	
AGC	Ser	10
TCA	Ser	
TCT	Ser	
ACT	Thr	
TGC	Cys	
AAC	Asn	വ
ATG	Met	
TCC	Ser	
GTC	Val	
GAT	Asp	, ←1
Ø		

TIG Leu Leu CTCGTC Val Pro CCI GGT Gly 25 GAA Glu GGG Gly GAC CCT Asp Pro CAG Glu AAG Lys TGG CTA

CAG Gln ACT Leu AGA Arg GGA Gly AAT Asn TCA ACC GAA TTG GGT Ala 35 Lys AAG Tyr TTA

TCC Ala GCA Ser Ile GAC AGC TTC CTG AAT Asp Ser Phe Leu Asn AGA AAG Arg Lys ACC ATA Ile Gly TTT Phe

 $_{\rm Gly}$ GCC CTC ACC Ala Leu Thr CAG Gln **GGG G1y** TTC TGT GCT Phe Cys Ala TAC TYF 70 ATC Ile 66C 61y GTA Val GAT Asp Ser AGT Pro

ATT CCA Ile Pro GTC TTG ACG (Leu Thr Leu ACA AGT Thr Ser ცვც Gly ACA Thr 666 G1y 85 TAT TTT Tyr Phe Phe CAG AAC Asn

Ser Lys Asp GAC GTG TAC CAG CTG AGA Val Tyr Gln Leu Arg Ala CCT GCC Pro Pro Asp I CCT GAC AAC Asn

AGT GAC AA(

17. Patient 4, Clone α17.6 (8ΕΩ ID NO:31) Family Specific Primer Vα17

Gln Ser Pro 15 AGC CCT CAG Glu GAG Gln Leu Val CAG CTG GTG TCT Ser GTC Val Arg AGA Phe GGA ACT Val Leu CTT

Cys Ala 30 TGT GCT Asn ATT ATA AAC Ile Ile TCA Ile Ser ATT Gly 999 GGA Gly Lys GTC CAG AAA Val Gln Lys Ile $\mathbf{T}\mathbf{T}\mathbf{G}$ Leu \mathtt{TCT} Ser

Pro CCI Trc Gln CAA CAA Gln TAC Tyr TGG Trp CCA Pro Phe TTT40 TAC Tyr GCG TTT GAC Ala Phe Asp Thr AAC Asn GAG

Lys GAA Glu AGT Ser GTG Val GAT Asp 60 Pro CCA Arg ATA CGT Ile ATA GCC Ile Ala Len \mathbf{TTG} TTA Leu Ala GCA Pro CCT GGC AAA Lys

Phe TTC Gln CAG Lys GCC AAG Ala Ser TTC AAT AAA AGT Lys Phe Asn ATC TCC ACA \mathtt{Thr} TTC AGA Arg Gly GAA AAA Lys 65 TTC Phe Tyr ACC Thr Ala Ser GAC TCA Asp G1y 90 GGA Pro CAG CCT Gln TCC Ser GAT Asp Met ATC ATG Ile CAT Leu TTGTCA

Leu GAG Thr 110 ACG GGA Gln Gly CAG TTC GGA (Phe Gly (ATC Ile GGA AAG CTT Gly Lys Leu GGA Gly Glu GAG GCA Ala TGT

Crg	
CAG Gln	
TAC Tyr	
GTG Val 125	
GCC Ala	
CCT	
GAC Asp	
CCT	
AAC Asn 120	AAG Lys
CAG Gln	GAC Asp 135
ATC Ile	AGT
AAT Asn	TCC
CCC Pro	AAA Lys
AAA Lys 115	TCT Ser
GTG Val	GAC ASP
TCT	AGA Arg

Antibodies may be developed against the T cell receptors or against amino acid sequences and portions thereof, corresponding to said T cell receptor variable regions such as those set forth in Table 1 for commercial purposes by developing monoclonal antibodies as indicated These murine or rat or other herein and known in the art. administered directly. be monoclonals could species Alternatively, to reduce xenogeneic responses monoclonals, these antibodies can be "humanized" by grafting a human constant region onto the non-human variable region, or by transplanting the non-human hypervariable regions onto a human antibody. (50, 51) Polyclonal antibodies can also be employed, particularly if they are from a species which exhibits little immunogenicity in humans such as pigs. Antigenic fragments may be derived from family-specific sequences such as those contained in the variable region primers or from hypervariable regions as defined in Jones et

al. (52) The association of RA with HLA-is reminiscent of models experimental in seen associations similar autoimmune experimental as such autoimmunity, encephalomyelitis, a model for multiple sclerosis triggered by autoreactive T cells reactive to myelin basic protein and specific MHC Class II antigens (9-12). The observation of a restriction to certain MHCs in such experimental systems correlates with a restricted repertoire of T cell antigen receptors which respond to that MHC + antigen (13). This has also been documented in multiple sclerosis T cell lines derived from humans (14, 15). In experimental systems, antibodies directed to the relevant T cell receptors, or immunization with peptides derived from these T cell receptors, is capable of ameliorating the disease (10, 11).

In another embodiment of the invention a method of treating rheumatoid arthritis in a mammal is provided which comprises administering to said mammal an effective amount of antibodies to mammalian T cell receptor variable regions selected from the group consisting of Val7, Val, V β 12, V β 14,

凄

 $V\beta$ 17 and $V\beta$ 7 and antigenic fragments thereof. In particular, antibodies to amino acid set forth in Table 1 and portions thereof are preferred.

Antibodies to mammalian T cell receptor variable regions selected from the group consisting of Val7, Val, V β 12, V β 14, V β 17 and V β 7 and antigenic fragments thereof can be prepared as described above.

An effective amount of antibodies to at least one of the T cell receptor variable regions described above is then administered to the mammal. An effective amount of antibodies is that amount which reduces the level of T cells bearing the corresponding receptor in the synovium or which results in clinical signs of improvement in the patient.

Of course the method of treating rheumatoid arthritis of the present invention may be combined with other traditional treatments for the disease where indicated.

It is believed the therapy of the invention could be administered at any point in the course of rheumatoid arthritis.

A method for immunizing a mammal to prevent the occurrence of rheumatoid arthritis or to ameliorate active disease is also provided by the invention. The method comprises administering to said mammal mammalian T cell receptor variable regions selected from the group consisting of Val7, Val, V β 12, V β 17, V β 7 and antigenic fragments thereof. Amino acid sequences as set forth in Table 1, and portions thereof, are preferred for some embodiments of the invention.

Mammals could be immunized by using the T cell receptor variable regions described above and antigenic fragments thereof, with or without agents known to those in the art attached thereto to increase the antigenic potential Generally the antigen or protein can be of the antigen. dissolved at between about $1\mu g/ml$ to about 1g/ml in sterile saline or saline with 0.4 mg aluminum hydroxide per ml as a vehicle. Generally 0.5 to 1.0 ml of the protein solution is and then followed by booster injected intramuscularly months after the initial one and 6-12 injections at

immunization. An effective amount is that amount of antigen sufficient to raise antibodies to the antigen in the animal.

There is precedence for immunizing mammals with T cell receptor variable regions as protection against experimental autoimmune encephalomyelitis. (11, 12) It is believed that a patient to be immunized would either have clinical evidence of rheumatoid arthritis, have a strong family history of rheumatoid arthritis or have the genetic predisposition for rheumatoid arthritis described herein.

Kits with the antibodies described herein useful in the treatment of rheumatoid arthritis or kits with antigens for immunization are also within the scope of this invention.

Materials and Meth ds

Synovial tissu and Cell Lines: Tissue was obtained at the time of joint surgery, and was handled sterily at all times. The tissue was rinsed in sterile phosphate buffered saline (PBS), placed in a petri dish, the superficial layer snipped off with scissors and minced with a sterile scalpel. minced tissue was placed in 20 mls PBS with 5% HEPES buffer, 0.4 g hyaluronidase (type 1-S), 0.04 g DNA-ase 1 (type II from bovine pancrease) and 1.2 g collagenase (Type Z) (all from Sigma, St. Louis, MO) with 1% fetal calf serum (FCS), and stirred continuously for 90 minutes at 37°C. The large chunks of tissue were decanted, and the cells centrifuged and washed twice in culture media (RPMI 1640 with pen/step, L-glutamine, sodium pyruvate, non-essential amino acids, HEPES buffer 5X10 5 M β -mercaptoethanol (all from Gibco, Gaithersburg MD), and 10% FCS (Hyclone). The T cells were purified by standard nylon wool chromatography (17), cultured overnight at $1 \times 10^6/ml$ in culture media, and the non-adherent cells separated, centrifuged, and maintained in culture. Stimulation of the cells was with either phytohemagglutinin (1% solution, from Sigma), interleukin-2 (Amgen Biologicals, Thousand Oaks, CA), or media alone. Cells were stimulated for 3-5 days, and then maintained for varying periods of time in 10 U/ml IL-2 prior to analysis.

Fluorescence-Activated Cell Sorter (FACS) Analysis:

Following culture, cells were centrifuged, washed and resuspended in FACS media (1% bovine serum albumin in PBS with 0.1% sodium azide), at 1x10⁶ cells per 100µl. Primary antibody was added for 20-40 minutes on ice. After an additional two washings, the cells were subjected to second antibody (fluorescein isothiocyanate-conjugated goat anti-mouse Ig (Sigma); at 1:100 dilution), then washed twice again. The cells were then analyzed at the University of Pennsylvania Cancer Center FACS facility. Per cent positive was determined by comparing the samples to a no primary antibody control. Antibodies used were OKT3 anti-CD3 (Ortho Diagnostics,

Raritan, NJ), Leu3a anti-CD4 (Becton-Dickinson **provid** location), and OKT8 (Ortho), at the dilutions suggested by the suppliers.

RNA Extraction and cDNA Synthesis: Tissue was homogenized in isothiocyanate (GITC) solution, or quanidinum resuspended in GITC solution, and vortexed for 30 seconds. 0.1 ml 2 M sodium acetate pH 4 was added, the solution vortexed, followed by 1.0 ml diethylpyrocarbonate (DEP)-water-saturated phenol, the sample mixed, then 0.2 ml phenylisoamyl alcohol, thorough vortexing, and the solution transferred to sterile EPPENDORF tubes. Each sample was then incubated on ice for 20 minutes, microfuged for 10 minutes, and the top layer recovered, RNA precipitated with 2.5 volumes of 100% ethanol and 1/10 volume 1M sodium acetate pH 5.5 in dry ice/ethanol for 30 minutes. The solutions were microfuged for 15 minutes, the supernatant decanted, the pellets washed in 70% ethanol and rotary evaporated. The dried pellets were resuspended in 50 μ l DEP-water and RNA quantitated spectrophotometrically.

For reverse transcription, 1-20 μ g of RNA in 10 μ l was utilized to synthesize cDNA primed with random hexamers in the following reaction mixture: $3\mu 1$ Maloney Murine Leukemia Virus reverse transcriptase with 6 μ l 5x reverse transcriptase buffer, 1.5 μ l RNAse inhibitor, and 3 μ l 0.1 M dithiothreitol (all from GIBCO/BRL, Gaithersburg, MD), 3 μ l random hexamers (from Pharmacia LKB Biotechnology, Piscataway, NJ), and either 1 or 3 μ l 100 $\underline{m}\underline{M}$ dNTPs (25 $\underline{m}\underline{M}$ in each dNTP, from Boehringer 10 minute Following a Germany). GmbH W. Mannheim, preincubation at 25°C, the reaction was carried out for 1 hour at 42°C, then 95°C for 5 minutes followed by storage at -20°C until use.

PCR Amplification T Cell Receptor Variable Regions: cDNA was amplified utilizing the primers listed in Figure 5 with $Va/\beta n$ and Ca/β_{mid} at 0.2 nM concentrations. cDNA was amplified utilizing Thermus aquaticus DNA polymerase (Taq polymerase) and standard reaction conditions suggested by the manufacturer

ŧ

. 🔮

(Perkin-Elmer Cetus Corp., Norwalk, CT). The reaction mixture contained 10 μ l of 10X reaction buffer, 16 μ l 1.25 $\underline{\text{nM}}$ dNTPs (final concentration 200 μM in each dNTP), 5 μl of each oligonucleotide primer at 20 μM (final 1 μM in each primer), 5 μ l of DNA, 0.5 μ l of DNA, 0.5 μ l Taq polymerase, and 58.5 ml distilled/deionized water. Primers were synthesized by the Wistar Institute oligonucleotide synthesis facility. program utilized 5 initial low temperature cycles for low stringency (95°C for 1 min., 37°C for 2 min., 52°C for 2 min.), followed by higher stringency for 40 cycles (95°C for 1 min., 52°C for 2 min., 72°C for 2 min), and a final 5 minute 72°C elongation phase. For some experiments, the initial 20 cycles, described above, was used followed by additional increments of 5 higher stringency cycles (95°C for 1 min., 52°C for 2 min., 72°C for 2 min), with PCR product removed following each increment of 5 cycles for analysis. Products were analyzed by electrophoresis on 2-3% agarose gels stained with ethidium bromide.

Determination of Sequences of T Cell Receptor Variable Regions: PCR products were cloned into the TA cloning vector (InVitrogen, San Diego, CA) according to kit instructions. Plasmid DNA was isolated from the clones as described by Ausubel, et al., Current Protocols in Molecular Biology (John Wiley & Sons, New York, NY) and Sambrook, et al., Molecular Cloning. A Laboratory Manual (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.) incorporated by reference in their entireties. A portion of the cloned cDNA was sequenced in accordance with methods provided by Ausubel, et al., Current Protocols in Molecular Biology (John Wiley & Sons, New York, NY) and Sambrook, et al., Molecular Cloning. Laboratory Manual (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.) incorporated by reference in their entireti s. Amino acid sequences were determined as set forth in Table 1. Relative positions set forth in Table 2 were determined in relation to family specific variable region primers used and published data providing invariant residues

TABLE II

RHEUMATOID SYNOVIAL T CELL RECEPTOR ALPHA CHAINS

							34	1/1										
					,	70		LRCNYSYGAT.PYLFWYVQSPGQGLQLPLKYFSGDTLVQGI	LRCNYSYGAT, PYLFWYVQSPGQGLQLLLKYFSGDTLVQGI	LRCNYSYSGS.PELFWYVQYSRQRLQLLLRHISRES,IKG	LSLDLESLSCSYTVSGLRGL.FWYRQDPGKGP.EFLFTLYSAGEEKEK	LSLDLESLSCSYTVSGLRGL. FWYRQDPGKGP. EFLFTLYSAGEEKEK	LSLDLESLSCSYTVSGLRGL. FWYRODPGKGP. EFLFTLYSAGEEKEK	L.SLDLESLSCSYTVSGLRWL.FWYRQDPGKGP.EFLFALYSAGKEKEK	DVSMNCTSSSIFNTWLWYKQDPGEGPVLLIALYKAGELTSNG	VSQLVEQSPQSLIVQKGGISIINCAYENTAFDYPP WYQQFPGKGPA LLIAIRPDVSEKKE		
				•		09		SPGQGLQ	SPGQGLQ	QYSRQRI	DPGKGP.	DPGKGP.	DPGKGP.	DPGKGP.	DPGEGP) FPGKGP!		
WYRQ	LVK	HKH	1	Ø				WYVQ	ŎΛXΜ,	FWYV	WYRC	WYRC	WYRC	WYRG	.WYKÇ	MXOC		
	•	•	•	•		20		T. PYLF	T. PYLF	GS.PEI	LRGL. F	LRGL. F	LRGL.	SLRWL.	NTWL.	AFDYPP		
								SYGA	SYGA	IYSYS	TVSG	TVSG	TVSG	TVSC	SSSIE	KENT?		
CN	H	ß	Ą	Ω		40		LRCNY	LRCNY	LRCN	SLSCS	SLSCS	SISCS	SISCS	SMNCT	IINCA		
											TDLE	LDLE	LDLE	LDLE	DV	KGGIS		
团						30					31	17	77	ä		TIVO		
																30SPQ		
						20		15)			_	_	_	_	~	SQLVE		
					de)				:17)	:19)	0:25	NO:23	NO:21	NO:27	10:29	LVTGFRV		
					epti	10	_	ΔI ζ	ED NO	Clone a1.4 (SEQ ID NO:19)	N QI	ID N	ID N	ID N	(SEQ ID NO:29	LVJ	_	
					der I			(SE	SEQ	SEQ	(SEO	(SEQ	(SEQ	(SEQ ID			10:31	
					Lea			1.1/2	1.3 (1.4 (17.1	17.2	17.3	17.4	17.5	17.6	(SEQ ID NO:31)	
					ludes	₩.	<u></u>	ne α]	ne α΄.	ne a	ne g	ne a	ne a	ne α	ne a	Clone ¤17.6	(SEQ	
					(Inc			C10	clo	Clo	Clo	Clo	C10	1 010	, clc			
RVED	UES				NOI			nt 1	int 2	int 2	int 2	int 3	ent 4	ent 4	ent 7	ent 4		
CONSERVED	RESIDUES				POSITION (Includes Leader Peptide)			Patient 1 Clone α1.1/2 (SEQ ID NO:	Patient 2 Clone al.3 (SEQ ID NO:17)	Patient 2	Patient 2 Clone α17.1 (SEQ ID NO:25	Patient 3 Clone $\alpha 17.2$ (SEQ ID	Patient 4 Clone α17.3 (SEQ ID	Patient 4 Clone ¤17.4	Patient 7 Clone ¤17.5	Patient 4		
J	-				-			. •					SL	JB	ST	IT	JTE	=

SUBSTITUTE SHEET

34/2

RHEUMATOID SYNOVIAL T CELL RECEPTOR BETA CHAINS	OVIAL	T CELI	RECEPTO	R BETA CHAI	S		
CONSERVED RESIDUES V Q P	н	ტ	ပ	FWYQQ	ı		
SI	Λ	E		Y RK	Ь		
				¥	v		
POSITION (Includes Leader Peptide)							
1 10 20	30		40	50	09	70	
					_		
Patient 6 Clone β 14.1 (SEQ ID NO:1)						Δ	VT.DKG
Patient 6 Clone β 14.2 (SEQ ID NO:3)						Δ	VT.DKG
Patient 5 Clone β 14.3/4/5/6 (SEQ ID NO:5)	5)					H	ET. DKG
Patient 5 Clone β 14.7 (SE ID NO:7)						Λ	VT.DKG
Patient 5 Clone eta 14.8 (SEQ ID NO:9)						Λ	VT.DKG
Patient 6 Clone eta 17.1 (SEQ ID NO:11)						Έų	FQ. KG
Patient 6 Clone eta 17.2 (SEQ ID NO:13)						Œι	FQKG
Patient 6 Clone eta 17.3 (SEQ ID NO:33)						£4	FQ. KG

SUBSTITUTE SHEET

TABLE II

RHEUMATOID SYNOVIAL T CELL RECEPTOR ALPHA CHAINS

CONSERVED			ſΞŧ	DS	YFCA	FG
RESIDUES			н	Ŧ	Y G	A
			ı	A	ΓΛ	
			Λ		н	
			ß			
POSITION (Includes Leader Peptide)	de)					
		. 08	90 1	100	110	120
			_	_		
Patient 1 Clone al.1/2 (SEQ ID NO: 15) KGFEAEFKRSQSSFNLRKPSVHWSDAAEYFCAVGADSGYSTLTFGKG	NO: 15)	KGFEAEFKR	SQSSFNLRKPS	SVHWSDAA	EYFCAVGAI	SGYSTLTFGKG
Patient 2 Clone al.3 (SEQ ID NO:17)	:17)	KGFEAEFKR	SQSSFNLRKP	SVHWSDAA	EYFCAVGP	KGFEAEFKRSQSSFNLRKPSVHWSDAAEYFCAVGPTHNDMRFGAG
Patient 2 Clone α1.4 (SEQ ID NO:19)	:19)	FTADLNKGE	rsfhlkki	PFAQEEDS	AMYYCALA	FTADLNKGETSFHLKKPFAQEEDSAMYYCALALQATSLLLEEEP
Patient 2 Clone $lpha$ 17.1 (SEQ ID N	NO:25)	ERLKATLTK	KESFLHITAP	KPEDSA	TYLCAVRRSDG	ERLKATLTKKESFLHITAPKPEDSATYLCAVRRSDGQKLLFARGTMLK
(SEQ ID	NO:23)	ERLKATLTK	KESFLHITAP	KPEDSA	TYLCAVRRSDG	ERLKATLTKKESFLHITAPKPEDSATYLCAVRRSDGQKLLFARGTMLK
Patient 4 Clone α17.3 (SEQ ID N	NO:21)	ERLKATLTK	KESFLHITAP	KPEDSA	TYLCAARRSDG	ERLKATLTKKESFLHITAPKPEDSATYLCAARRSDGQKLLFARGTMLK
Patient 4 Clone α17.4 (SEQ ID N	NO:27) .	ERLKATLTK	KESFLHITAP	KPEDSA	TYLCAVRRSDG	ERLKATLTKKESFLHITAPKPEDSATYLCAVRRSDGQKLLFARGTMLK
Patient 7 Clone α 17.5 (SEQ ID N	NO:29)	RLTAQFGIT	RKDSFLNISA	SIP.SDVG	IYFCAGQALTG	RLTAQFGITRKDSFLNISASIP.SDVGIYFCAGQALTGNQFYFGTGTSLT
Patient 4 Clone a17.6 (SEQ ID N	ID NO:31)	GRFTISFNK	SAKQFSLHIM	DSQPGDSA	TYFCAAEGGKL	GRFTISFNKSAKQFSLHIMDSQPGDSATYFCAAEGGKLIFGQGTELS

RHEUMATOID SYNOVIAL T CELL RECEPTOR BETA CHAINS

CONSERVED RESIDUES POSITION (Includes Leader Peptide) Patient 6 Clone β 14.1 (SEQ ID NO:1) Patient 6 Clone β 14.2 (SEQ ID NO:3) Patient 5 Clone β 14.3/4/5/6 (SEQ ID NO:5) Patient 5 Clone β 14.7 (SE ID NO:7) Patient 5 Clone β 14.8 (SEQ ID NO:9) Patient 6 Clone β 17.1 (SEQ ID NO:11) Patient 6 Clone β 17.2 (SEQ ID NO:113) DIAH	L DSS YLCAS M QT F SA H G O T V N N N N N N N N N N N N	100 100 LESASTN LESASTN LESASTN LESASTN LESASTN LESASTN LESASTN	DSS YLCAS QT F SA H G O T V 110 QTSMYLCASSSQKP IQTSMYLCASSLLQR IQTSMYLCASSLLQR IQTSMYLCASSLLQR IQTSMYLCASSLLQR IQTSMYLCASSLLQR
7 - 1 - 1 - 1	GORDON TO THE TOTAL TO THE TOTAL TOT	MCOCOG I	מספרמס גיספע זספיס

SUBSTITUTE SHEET

34/5

TABLE II

ALPHA CHAINS
RHEUMATOID SYNOVIAL T CELL RECEPTOR ALPHA CHAINS
SYNOVIAL T
RHEUMATOID

CONSERVED	NIQ D Y	
POSITION (Includes Leader Peptide)	130 140 150	
Patient 1 Clone α 1.1/2 (SEQ ID NO: 15)	TMLLVSPDIQNPDPAVYQLRDSKSSDK TRLTVKPNIQNPDPAVYQLRDSKSSDK	KSSDK
Patient 2 Clone al.4 (SEQ ID NO:19)	. RVLVKPNIQNPDPAVYQLRDSKSSDK	KSSDK
Patient 2 Clone a17.1 (SEQ ID NO:25)	VDLNIQNPDPAVYQLRDSKSSDK	KSSDK
Patient 3 Clone a17.2 (SEQ ID NO:23)	VDLNIQNPDPAVYQLRDSKSSDK	KSSDK
Patient 4 Clone a17.3 (SEQ ID NO:21)	VDLNIQNPDPAVYQLRDSKSSDK	KSSDK
Patient 4 Clone $\alpha17.4$ (SEQ ID NO:27)	VDLNIQNPDPAVYQLRDSKSSDK	KSSDK
Patient 7 Clone a17.5 (SEQ ID NO:29)	VIPNIQNPDPAVYQLRDSKSSDK	KSSDK
Patient 4 Clone a17.6 (SEQ ID NO:31)	VKPNIQNPDPAVYQLRDSKSSDK	KSSDK

RHEUMATOID SYNOVIAL T CELL RECEPTOR BETA CHAINS

	FG G	120 130 140 150	 NSKTFGSGTRFSVVEDLNKVFPPEVAVFEPSEAE	. EAFFGQGTRLTVVEDLNKVFPPEVAVFEPSEAE	: 5) TTTDTQYFGPGTRLTVLEDLKNVFPPEIAVFEPSEAE	EQY FGP GTRLTVTEDLKNVFPPEVAVFEPSEAE	TDTQYFGPGTRLTVLEDLKNVFPPEVAVFEPSEAE	GLTGAKNIQYFGAGTRPSVLEDLKNVFPPEVAVFEPSEAE	YNEQFFGPGTRLTVLEDLKNVFPPEVAVFEPSEAE	ASYGYT FGS GTRLTVVEDLKNVFPPEVAVFEPSEAE
TOTALOUIC DIOTUNIONIN	CONSERVED RESIDUES	POSITION (Includes Leader Peptide)	Patient 6 Clone β 14.1 (SEQ ID NO:1)	Patient 6 Clone β 14.2 (SEQ ID NO:3)	Patient 5 Clone β 14.3/4/5/6 (SEQ ID NO:	Patient 5 Clone β 14.7 (SE ID NO:7)	Patient 5 Clone β 14.8 (SEQ ID NO:9)	Patient 6 Clone β 17.1 (SEQ ID NO:11)	Patient 6 Clone β 17.2 (SEQ ID NO:13)	Patient 6 Clone β 17.3 (SEQ ID NO:33)

and numbering of sequences of known T cell receptor regions. Kabat, E.A., et al., Sequences of Proteins of Immunological Interest" 4th ed., U.S. Department of Health and Human Services, Public Health Service National Institute (1987).

Transfer and Probing Agarose gels were transferred to nylon fibers (Genescreen Plus, Du Pont New England Nuclear, Boston, MA) by capillary transfer overnight. Hybridization was with Figure primers noted in Cβ5' Cα5′ or either Oligonucleotide labeling employed 100 ng DNA, 75 μ C; 32 P-ATP, 2.5 μ l 10 x kinase buffer (500 mM Tris HCL pH 7.6, 100 mM ${
m MgCL}_2$, 50 mM dithiothreitol, 1 mM spermadine, 1 mM EDTA), 10 U T4 DNA kinase adjusted to a final volume of 25 μ l with distilled water. Labelling was carried out by incubation at 37°C for 30 minutes prior to use. Blots were prehybridized in 5x SSC, 5x Denhardt's solution, 0.1% SDS for 1-1.5 hours at 55°C in sealable polyethylene bags, most of the solution poured off, ^{32}P -labelled oligonucleotide added (75 μ Ci) and hybridized for 2-3 hours at 42°C or overnight at 4°C, the blots washed 1x in 2x SSC, 0.1% SDS for 20 minutes at 45°C, then 3x in 5x SSC, 0.1% SDS for 20 minutes at 45°C, an exposed to Kodak XRP film at -70°C for 2-72 hours.

Statistics

The standard error of occurrence of each TCR V region family was calculated by the formulae:

100 times the square root of (p[1-p]/n)

where "n" is the number of samples analyzed, and "p" is the number of positives. The frequency of occurrence of a particular TCR V region family was considered significantly increased if it was ≥ 2 standard errors higher than the mean for all V regions of that type (α versus β).

Results

PCR Prim rs

Primers derived from the human TCR alpha and beta constant regions were utilized in conjunction with primers specific for individual variable region families. (14-16). The primers utilized in these studies are listed in Fig. 5, and their relative positions on the coding strand of cDNA indicated in Figure 1. The constant region primers were designed as antisense primers to allow their use to prime both PCR reactions as well as probes for blotting. Variable region primers were designed to act in a family specific manner as has been previously reported (14-16).

The PCR program used in these studies employed a low stringency initial 5 cycles, followed by 40 cycles at higher stringency. The rationale for using this program was twofold. As these studies were designed to investigate the range of T cell receptors expressed in RA synovium, and all TCR V regions have not yet been sequenced, related TCR families which have sequences related to the primers used here may also be amplified in the initial low stringency cycles. 40 cycles of amplification were then used to amplify even low frequency transcripts. This should help overcome the potential problem of sampling error, which is possible from surgical specimens. Thus, if local accumulations of specific TCR bearing T cells are present, and such a local accumulation is missed in the surgical specimen, their presence still may be detected if they are also present at lower frequency in the surgical specimen examined. Preliminary experiments with these primers utilizing the program described in Materials and Methods revealed that all of them (except $V\beta16$) are effective in amplifying TCR V regions from PHA stimulated peripheral blood mononuclear cells, but that only the appropriate V region (primers amplified Jurkat cell cDNA TCR ((20) and data not shown).

Synovial T Cells RNA was extracted and cDNA synthesized from both whole synovium and PHA stimulated/IL-2-maintained synovial T cell lines. Synovial T cell lines derived in this

manner have been previously described (17), and early on represent a phenotypically mixed population, including CD8+ and CD4+ cells (17). FACS analysis was available for 4 of these cell lines at the time of analysis, and the data is shown in Table 3. In 3 of these, CD4+ cells predominated, while in the other, CD8+ cells were more prevalent.

PATIENT	PHENOTYPE OF CONTROL	TABLE 3 SYNOVIAL TISSUE T CD3+	CELL LINES CD4+	CD8+
EP ₁	1%	90%	25%	54%
NJ ₁	1%	80%	81%	9%
MW ₁	1%	99%	77%	28%
HR ₁	3%	98%	74%	15%

T cell receptor transcripts were amplified from cDNA derived from rheumatoid synovial T cell lines. All rheumatoid synovia were obtained at the time of joint surgery, and thus represented late disease. cDNA was split into equal portions and amplified with the middle constant region primers (C $eta_{ exttt{mid}}$ or $C\alpha_{mid}$) in combination with each of the respective individual variable region primers noted in Fig. 5 (eg., $C\beta_{mid}+C\beta 1$, $C\beta_{mid}+C\beta_{2},...C\beta_{mid}+C\beta_{20};$ $C\alpha_{mid}+C\alpha_{1};$ $C\alpha_{mid}+C\alpha_{2},....C\alpha_{mid}$, $C\alpha_{18}$). Following electrophoresis and transfer, these were probed with $C\beta5'$ or $C\alpha5'$ respectively. The results for the synovial T cell lines is shown in Figure 2. An average of 15 alpha chain and 15.8 beta chain families were detected in these cell This suggests that a quite heterogeneous population of T cells is present in synovium. However, as these cell lines were initially expanded with PHA, it is possible that the proportion of the various TCR subsets alter during culture. In addition, the ability of PHA to activate resting T cells raises concern about the relative proportion of activated T cells following stimulation compared with prior Therefore, similar analyses were performed to stimulation. on whole, unstimulated rheumatoid synovium.

Rheumatoid Synovium

The results for the whole synovia or freshly isolated, unstimulated synovial T cells analyzed similarly are

shown in Figure 3. An average of 4.2 alpha chain and 9.7 beta chain families were detected by this technique. The intensity of the bands detected is quite variable in Figure 2 & 3. To further evaluate the technique, cDNA was pooled from 4 synovia, and amplified with these primers for increasing numbers of cycles (Figure 4). Note that the intensity of some bands which appeared in early cycles faded relative to the intensity of bands which arose at later cycles. Thus, the intensity of the bands cannot be taken as an indicator of their relative abundance.

The frequency of occurrence of each TCR variable region was tabulated for synovial tissue in Figures 6 and 7. While the T cell receptor expression seen in the synovial T cell lines is quite heterogeneous, the expression in rheumatoid synovia was somewhat more limited. Specifically, Va17 was present 7/10 synovia, and Va1 was present in 5/10. V β 14 was seen in 9/10 samples, while V β 17 and V β 12 were present in 8/10 specimens and V β 7 was seen in 7/10. This suggests the presence of these variable regions in many rheumatoid synovia from many different patients. When analyzed statistically, the frequency of V β 12, 14 & 17 were \geq 2 standard errors above the mean values for all TCR V β s detected, and Va17 and \geq 2 standard errors above the mean values for all TCR V α a detected.

Ref renc s

- 1. Firestein, G., and N. Zvaifler, 1987. The pathogenesis of rheumatoid arthritis. *Immunology of rheumatic diseases* 13:447-461.
- 2. Duke, O., G.S. Panayi, G. Janossy, and L. W. Poulter, 1982. An immunohistological analysis of lymphocyte subpopulations and their microenvironment in the synovial membranes of patients with rheumatoid arthritis using monoclonal antibodies. Clin Ex. Immunol 49:22-30.
- 3. Gao, X., E. Ball, L. Dombrausky, N. Olsen, T. Pincus, M. Kahn, and F. Wolfe, 1988. Class II human leukocyte antigen genes and T cell receptor polymorphisms in patients with rheumatoid arthritis. Am J. Med 85:14-16.
- 4. Goronzy, J., C. Weyand, and C. Fathman, 1986, Shared T cell recognition sites on human histocompatibility antigen class II molecules of patients with seropositive rheumatoid arthritis. J. Clin Invest 77: 1042-1049.
- 5. Gregerson, P., J. Silver, and R. Winchester, 1988. Genetic susceptibility to rheumatoid arthritis and human leukocyte antigen polymorphism. The role of shared conformational determinants. Am J Med 85: 17-19.
- 6. Merryman, P., R. Crapper, S. Lee, P. Gregersen, and R. Winchester. 1989. Class II major histocompatibility complex gene sequences in rheumatoid arthritis. Arthritis Rheum 32:251-258.
- 7. Nepom, G., J. Hansen, and B. Nepom. 1987. The molecular basis for HLA class II association with rheumatoid arthritis. J Clin Immunol 7:1-7.
- 8. Roudier, J., J. Petersen, G. Rhodes, J. Luke, and D. Carson, 1989. Susceptibility to rheumatoid arthritis maps to a T-cell epitope shared by the HLA-Dw4 DR β -1 chain and the Epstein-Barr virus glycoprotein gp 110. Proc Natl Acad Sci USA 86:5104-5108.
- 9. Saki, K., A.A. Sinha, D.J. Mitchell, S.S. Zamvil, J.B. Rothbard, H.O. McDevitt, and L. Steinman. 1988. Involvement of distinct murine T-cell receptors in the autoimmune encephalitogenic responses to nested epitopes of myelin basic protein. *Proc Natl Acad Sci USA* 85:8608-8612.
- 10. Hashim, G., A. Vandenbark, A. Galang, T. Diamanduros, E. Carvalho, J. Srinivasan, R. Jones, M. Vainiene, W. Morrison, and H. Offner. 1990. Antibodies specific for $V\beta 8$ receptor peptide suppress experimental autoimmune encephalomyelitis. J Immunol 144:4621-4627.

- 11. Vandenbark, A.G. Hashim, and H. Offner. 1989. Immunization with a synthetic T-cell receptor V-region peptide protects against experimental autoimmune encephalomyelitis. Nature 341:541-544.
- 12. Beraud, E., T. Resshef, A.A> Vandenbark, H.Offner, R. Fritz, C.H.J. Chou, D. Brnard, and I.R. Cohen, 1986. Experimental autoimmune encephalomyelitis mediated by T lymphocyte lines: Genotype of antigen-presenting cells influences immunodominant epitope of basic protein. J Immunol 136:511-515.
- 13. Burns, F.R., X. Li, N. Shen, H. Offner, Y.K. Chou, A.A. Vandenbark, and E. Heber-Katz. 1989. Both rat and mouse T cell receptors specific for the encephalitogenic determinant of myelin basic protein use similar $V\alpha$ and $V\beta$ chain genes even though the major histocompatibility complex and encephalitogenic determinants being recognized are different. J Exp Med 169:27-39.
- 14. Oksenberg, J.R., S. Stuart, A.B. Beegovich, R.B. Bell, H.A. Erlich, L.Steinman, and C.C. A. Bernard, 1990. Limited heterogeneity of rearranged T-cell receptor $V\alpha$ transcripts in brains of multiple sclerosis patients. 345:344-346.
- 15. Wucherpfennig, K.W., K. Ota, N.Endo, J.G. Seidman, A. Rosenzweig, H.L. Weiner, and D.A. Hafler, 1990. Shared human T cell receptor $V\beta$ usage to immunodominant regions of myelin basic protein, 248:1016-1019.
- 16. Choi. Y., B. Kotzin, L. Herron, J. Callahan, P. Marrack, and J. Kappler, 1989. Interaction of Staphylococcus aureus toxin superantigens with human T cells. Proc Natl Acad Sci USA 86:8941-8945.
- 17. Santoli, D., P. Phillips, T. Colt, and R. Zurier, 1990. Suppression of interleukin-2 dependent human T cell growth in vitro by prostaglandin E (PGE) and their precursor fatty acids in vitro. J Clin Invest 85:424-432.
- 18. Ausubel, R., R. Brent, R. Kingston, D. Moore, J. Seidman, J. Smith, and K. Struhl. 1989. Current Protocols in Molecular Biology. Greene Publishing Associates and Wiley-Interscience, John Wiley & Sons, New York, N.Y.
- 19. Sambrook, J. E. Fritch, and T. Maniatis. 1989. Molecular Cloning. A Laboratory Manual. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
- 20. Williams, W.V., A. Sato, M. Rossman, Q. Fang, and D. B. Weiner, (submitted). Semi-random DNA amplification utilizing the polymerase chain reaction. Application to the analysis of antigen receptor variable regions.

- 21. Nako, H.K. Eguchi, A. Kawakami, K. Migita, T. Otsubo, U.Y., C. Shimomura, H. Tezuka, M. Matsunaga, K. Maeda, and e. al. 1990. Phenotypic characterization of lymphocytes infiltrating synovial tissue from patients with rheumatoid arthritis: analysis of lymphocytes isolated from minced synovial tissue by dual immunofluorescent staining. J. Rheumatol 17:142-148.
- 22. Ranki, A., T. Paavonen, E. Tolvanen, U. Kankaanpaa, and P. Hayry. 1984. T lymphocyte subclasses in rheumatoid synovia as analyzed with monoclonal antibodies and functional in vitro tests. Scand J. rheumatol 13:67-76.
- 23. Lapadula, G., M. Covelli, R. Numo, G. Tricarico, G. Amendoni, and C. Berlingerio. 1984. Monoclonal antibody investigation in rheumatoid arthritis: presence of a T cell subpopulation bearing a double marker. Clin Rheumatol 3:137-144.
- 24. Holmdahl, R., L. Klareskog, K. Rubin, E. larsson, and H. Wigzell. 1985. Tlymphocytes in collagen II-induced arthritis in mice. Characterization of arthritogenic collagen II-specific T-cell lines and clones. Scand J Immunol 22:295-306.
- 25. Holoshitz, J., Y. Naparstek, A. Ben-Nun, and I. R. Cohen. 1983. Lines of T lymphocytes induce or vaccinate against autoimmune arthritis. *Science* 219:56-58.
- 26. Holoshitz, J., A. Matitiau, and I. R. Cohen, 1984. Arthritis induced in rats by cloned T lymphocytes responsive to mycobacteria but not to collagen type II. *J Clin Invest* 73:211-215.
- 27. van Eden, W., J. Holoshitz, Z. Nevo, A. Frenkel, A. Klajman, and I.R. Cohen, 1985. Arthritis induced by a T-lymphocyte clone that responds to Mycobacterium tuberculosis and to cartilage proteoglycans. Proc Natl Acad Sci USA 82:5117-5120.
- 28. Yoshino, S., E. Schlipkoter, R. Kinne, T. Hunig, and F. Emmrich, 1990. Suppression and prevention of adjuvant arthritis in rats by a monoclonal antibody to the alpha/beta T cell receptor. Eur J Immunol 20:2805-2808.
- 29. Clayton, J.P., G.M. Gammon, D.G. Ando, D.H. Kono, L. Hood, and E.E. Sercarz, 1989. Peptide-specific prevention of experimental allergic encephalomyelitis. Neonatal tolerance induced to the dominant T cell determinant of myelin basic protein. J. Exp Med 169:1681-1691.

200

4.5

- 30. Donoso, L.A., C.F. Merryman, T. Shinohara, B. Dietzschold, G. Wistow, C. Craft, W. Morley, and R. T. Henry, 1986. S-antigen; identification of the MAbA9-C6 monoclonal antibody binding site and the uveitopathogenic sites. Curr Eye Res 5:995:1003.
- 31. Donoso, LA., C.F. Merryman, T. Shinohara, T.W. Sery, and A. Smith, 1987. S-ANtigen. Experimental autoimmune uveitis following immunization with a small synthetic peptide. Arch Opthalmol 105:838-843.
- 32. Lennon, V.A., D.J. McCormick, E.H. Lambert, G.E. Griesmann, and M. Z. Atassi, 1985. Region of peptide 125-147 of acetylcholine receptor alpha subunit is exposed at neuromuscular junction and induces experimental autoimmune myasthenia gravis, T-cell immunity, and modulating autoantibodies. Proc Natl Acad Sci USA 82:8805-8809.
- 33. William, W.V., M. Kyriakos, G.C. Sharp, and H. Braley-Mullen. 1987. The cellular basis for the Ia restriction in murine experimental autoimmune thyroiditis. *Cellular Immunol*. 110:35-45.
- 34. Walker, C., C. Herzog, P. Rieber, G. Riethmuller, W. Muller, and W. J. Pichler. 1989. Anti-CD4 antibody treatment of patients with rheumatoid arthritis: II. Effect of in vivo treatment on in vitro proliferative response of CD4 cells. J Autoimmun 2:643:649.
- 35. Herzog, C., C. Walker, W. Muller, P. Rieber, C. Reiter, G. Riethmuller, P. Wassmer, H. Stockinger, O. Madic, and W.J. Pichler. 1989. Anti-CD4 antibody treatment of patients with rheumatoid arthritis: I. Effect on clinical course and circulating T cells. J Autoimmun 2:627-642.
- 36. Brahn, E., and D.E. Trentham, 1984. Effect of antihymocyte serum on collagen arthritis in rats: evidence that T cells are involved in its pathogenesis. *Cell Immunol* 86:421-428.
- 37. Schluesener, H., C. Brunner, K. Vass, and H. Lassmann, 1986, Therapy of rat autoimmune disease by a monoclonal antibody specific for T lymphoblasts. J. Immunol 137:3814-3820.
- 38. Goldschmidt, T.J., L. Jansson, and R. Holmdahl. 1990. In vivo elimination of T cell expressing specific T-cell receptor V beta chains in mice susceptible to collagen-induced arthritis. Immunology 69:508-514.
- 39. Kingsley, G. 1991. Monoclonal antibody treatment of rheumatoid arthritis. Br J Rheumatol Suppl 2:33-35.

- 40. Reiter, C., B. Kakavand, E.P. Rieber, M. Schattenkirchner, G. Riethmuller, and K. Kruger, 1991. Treatment of rheumatoid arthritis with monoclonal CD4 antibody M-T151. Clinical results and immunopharmacologic effects in an open study, including repeated administration. Arthritis Rheum 34:525-536.
- 41. Van Laar, J.M., A. Miltenburg, M. Vardonk, M. Daha, R.D. Vries, P.V.d. Elsen, and F. Breedveld. 1991. Lack of T cell oligoclonality in enzyme-digested synovial tissue and in synovial fluid in most patients with rheumatoid arthritis. Clin Exp Immunol 83:352-358.
- 42. Miltenburg, A., J.v. Laar, M. Daha, R.d. Vries, P.V.d. Elsen, and F. Breedveld. 1990. Dominant T-cell receptor betachain gene rearrangements indicate clonal expansion in the rheumatoid joint. Scand J. Immunol 31:121-126.
- 43. Duby, A., A. Sinclair, S. Osborne-Lawrence, W. Zeldes, L. Kan, and D. Fox. 1989. Clonal heterogeneity of synovial fluid T lymphocytes from patients with rheumatoid arthritis. Proc Natl Acad Sci USA 86:6206-6210.
- 44. Stamenkovic, I., M. Stegagno, K.A. Wright, S.M. Krane, E.P. Amento, R.B. Colvin, R.J. Duquesnoy, and J.T. Kurnick. 1988. Clonal dominance among T-lymphocyte infiltrates in arthritis. *Proc Natl Acad Sci USA* 85:1179-1183.
- 45. Sottini, A., L. Imberti, R. Gorla, R. Cattaneo, and D. Primi. 1991. Restricted Expression of T cell receptor V beta but not V alpha genes in rheumatoid arthritis. *Eur J. Immunol* 21:461-466.
- 46. Savill, C.M., P.J. Delves, D. Kioussis, P. Walker, P.M. Lydyard, B. Colaco, M. Shipley, and I.M. Roitt. 1987. A minority of patients with rheumatoid arthritis show a dominant rearrangement of T-cell receptor beta chain genes in synovial lymphocytes. Scand J Immunol 25:629-635.
- 47. Stamenkovic, I. M. Stegagno, K.A. Wright, S.M. Krane, E.P. Amento, R.B. Colvin, R.J. Duquesnoy and J.T. Kurnick. 1988. T lymphocyte infiltrates in inflammatory synovia are oligoclonal. *Transplant Proc* 20:315-319.
- 48. Paliard, X., S. West, J. Lafferty, J. Clements, J, Klapper, P. Marrack, and B. Kotzin. 1991. Evidence for the effects of a superantigen in rheumatoid arthritis. Science 253:325-329.
- 49. Devereux et al. "A comprehensive set of sequence analysis programs for the VAX", Nucleic Acids Research, 12:387-395 (1984).
- 50. Co et al., "Humanized antibodies for antiviral therapy", Proc. Natl. Acad. Sci.USA, **: 2869-2873 (1991).

- 51. Gorman, et al. "Reshaping a therapeutic CD4 antibody", Proc. Natl. Acad. Sci. USA, 88: 4181-4185 (1991).
- 52. Jones et al., "Resolution of hypervariabel regions in T-cell receptor β chains by a modified Wu-Kabat index of amino acid diversity", *Proc. Natl. Acad. Sci. USA*, 87: 9138-9142 (1990).

SEQUENCE LISTING

- (1) GENERAL INFORMATION:
 - (i) APPLICANT: Williams, William V. Weiner, David B.
 - (ii) TITLE OF INVENTION: T Cell Receptor-Based Therapy for Rheumatoid Arthritis
 - (iii) NUMBER OF SEQUENCES: 79
- (iv) CORRESPONDENCE ADDRESS: (A) ADDRESSEE: Woodcock Washburn Kurtz Mackiewicz and Norris
 - (B) STREET: One Liberty Place 46th Floor
 - (C) CITY: Philadelphia
 - (D) STATE: PA
 - (E) COUNTRY: U.S.A.
 - (F) ZIP: 19103
 - (V) COMPUTER READABLE FORM:
 - (A) MEDIUM TYPE: Floppy disk
 - (B) COMPUTER: IBM PC compatible
 - (C) OPERATING SYSTEM: PC-DOS/MS-DOS
 - (D) SOFTWARE: PatentIn Release #1.0, Version #1.25
 - (Vi) CURRENT APPLICATION DATA:
 - (A) APPLICATION NUMBER:
 - (B) FILING DATE:
 - (C) CLASSIFICATION:
 - (viii) ATTORNEY/AGENT INFORMATION:
 - (A) NAME: Johnson, Philip S.
 - (B) REGISTRATION NUMBER: 27,200
 - (ix) TELECOMMUNICATION INFORMATION:
 - (A) TELEPHONE: 215-568-3100
 - (B) TELEFAX: 215-568-3439

(2) INFORMATION FOR SEQ ID NO:1:

SEQUENCE CHARACTERISTICS

LENGTH: 237 base pairs (B)

TYPE: nucleic acid

STRANDEDNESS: double

TOPOLOGY: unknown

MOLECULE TYPE: DNA (genomic)

FEATURE: (ix)

(A) NAME/KEY: CDS

(B) LOCATION: 1..237

SEQUENCE DESCRIPTION: SEQ ID NO:1: (xi)

Arg 15 TCT Ser Val GAT AAG GGA GAT GTT CCT GAA GGG TAC AGT Asp Lys Gly Asp Val Pro Glu Gly Tyr Ser 5 Thr Val

GCC AGC ACC AAC CAG Ala Ser Thr Asn Gln ATT CTG GAG TCC Ile Leu Glu Ser CTG ATT CTG Leu Ile Leu TTC TCC Phe Ser Glu Arg 1 20 Lys AAG Lys AAG

CAA AAA CCC AAC AGT AAA Gln Lys Pro Asn Ser Lys Gln Lys Pro GCC AGC AGT TCA Ala Ser Ser Ser Ser Ser Leu Cys Tyr TAC Ser Met TCT ATG ACA Thr

Glu Asp Leu Asn Lys GAC CTG Val GTA Val TCC (Ser) TTG Thr Arg Leu 55 GGG ACC AGG Gly TCG Ser TTC GGT Phe Gly ACC

CCA TCA GAA GAG GTG TTT GAG GTC GCT သသ CCA TIC

Glu Pro Ser Glu Ala Val Phe Glu Val Ala Pro Pro

(2) INFORMATION FOR SEQ ID NO:2:

SEQUENCE CHARACTERISTICS: (ï,

(A) LENGTH: 79 amino acids

TYPE: amino acid **@**@

TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

G1uVal Thr Asp Lys Gly Asp Val Pro Glu Gly Tyr Ser Val Ser Arg 1 Thr Asn Gln Lys Lys Glu Arg Phe Ser Leu Ile Leu Glu Ser Ala Ser

Ser Met Tyr Leu Cys Ala Ser Ser Ser Gln Lys Pro Asn Ser Lys 35 45

Thr

Glu Asp Leu Asn Lys Thr Phe Gly Ser Gly Thr Arg Leu Ser Val Val 50

Val Phe Pro Pro Glu Val Ala Val Phe Glu Pro Ser Glu Ala Glu

(2) INFORMATION FOR SEQ ID NO:3:

SEQUENCE CHARACTERISTICS: (i)

LENGIH: 231 base pairs TYPE: nucleic acid

STRANDEDNESS: double

TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

FEATURE: (ix)

(A) NAME/KEY: CDS

LOCATION: 1..231 (B) (xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

Glu Ser AGT GTC Ser Val TAC GGG GTG ACT GAT AAG GGA GAT GTT CCT GAA Val Thr Asp Lys Gly Asp Val Pro Glu 1

Gln Asn ACC Ser GAG TCC GCC Glu Ser Ala GAG CGC TTC TCC CTG ATT CTG Glu Arg Phe Ser Leu Ile Leu 20 AAG GAG Lys AAG

Glu Ala GCT GAA TGG GGG ACT Trp Gly Thr Ser AGT AGC CTC TGT GCC Leu Cys Ala Tyr TAC ATG Met TCT Ser ACA

Glu Asp Leu Asn Lys Val Phe 60 AAC AAG CTG GAG GAC AGA CTC ACA GTT GTA Arg Leu Thr Val Val 55 ACC CAA GGC # Gln Gly 1 50

GAG GCA Glu Ala GAA GAG CCA TCA Phe Glu Pro Ser TTT GTGGCT Val GIC GAG Pro Pro Glu ပ္ပပ္ပ

(2) INFORMATION FOR SEQ ID NO:4

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 77 amino acids(B) TYPE: amino acid(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Val Thr Asp Lys Gly Asp Val Pro Glu Gly Tyr Ser Val Ser Arg Glu $_{\rm 1}$

Lys Lys Glu Arg Phe Ser Leu Ile Leu Glu Ser Ala Ser Thr Asn Gln 25

Ser Ser Trp Gly Thr Glu Ala Phe Phe 40 Thr Ser Met Tyr Leu Cys Ala

Gly Gln Gly Thr Arg Leu Thr Val Val Glu Asp Leu Asn Lys Val Phe

Glu Ala Glu Pro Pro Glu Val Ala Val Phe Glu Pro Ser

(2) INFORMATION FOR SEQ ID NO:5:

SEQUENCE CHARACTERISTICS: (i,

LENGTH: 246 base pairs TYPE: nucleic acid (B)

STRANDEDNESS: double

TOPOLOGY: unknown <u>(0</u>(2) (ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:

(A) NAME/KEY: CDS (B) LOCATION: 1...

LOCATION: 1..246

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

AGT GTC TCT AGA GAG Ser Val Ser Arg Glu 15 ACT GAT AAG GGA GAT GTT CCT GAA GGG TAC Thr Asp Lys Gly Asp Val Pro Glu Gly Tyr 5 GAG Glu

AAG GAG CGC TTC TCC CTG ATT CTG GAG TCC GCC AGC ACC AAC Lys Glu Arg Phe Ser Leu Ile Leu Glu Ser Ala Ser Thr Asn 20 AAG

GCC AGC AGT TTG CTC CAG CGG ACC ACA Ala Ser Ser Leu Leu Gln Arg Thr Thr 40 TAC

ACA GTG CTC GAG GAC Thr Val Leu Glu Asp CTG GGC CCA GGC ACC CGG Gly Pro Gly Thr Arg 55 Ser Met Tyr Leu Cys Ala Ser 35 Phe TTTT ACG CAG TAT 1. Thr Gln Tyr P. 50

Asp

GAT

Ser CTG AAA AAC GTG TTC CCA CCC GAG ATC GCT GTG TTT GAG CCA Leu Lys Asn Val Phe Pro Pro Glu Ile Ala Val Phe Glu Pro 65

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 82 amino acids

TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

Glu Thr Asp Lys Gly Asp Val Pro Glu Gly Tyr Ser Val Ser Arg Glu

Lys Lys Glu Arg Phe Ser Leu Ile Leu Glu Ser Ala Ser Thr Asn Gln 20

Thr Ser Met Tyr Leu Cys Ala Ser Ser Leu Leu Gln Arg Thr Thr 35 45

Asp Thr Gln Tyr Phe Gly Pro Gly Thr Arg Leu Thr Val Leu Glu Asp 50 55

Glu Leu Lys Asn Val Phe Pro Pro Glu Ile Ala Val Phe Glu Pro Ser 65

Ala Glu

(2) INFORMATION FOR SEQ ID NO:7:

SEQUENCE CHARACTERISTICS:

(A) LENGTH: 234 base pairs(B) TYPE: nucleic acid(C) STRANDEDNESS: double

TOPOLOGY: unknown

MOLECULE TYPE: DNA (genomic)

FEATURE: (ix)

(A) NAME/KEY: CDS (B) LOCATION: 1..234

SEQUENCE DESCRIPTION: SEQ ID NO:7: (xi)

GAG Glu	CAG Gln	TAC Tyr	GTG Val	
AGA Arg 15	AAC Asn	CAG Gln	AAC Asn	
TCT Ser	ACC Thr 30	GAG Glu	AAA Lys	GAG Glu
GTC Val	AGC	GGC Gly 45	CTG	GCA
AGT Ser	GCC Ala	AGG Arg	GAC Asp 60	GAA Glu
TAC Tyr	TCC	gac Asp	GAG Glu	TCA
GGG G1y 10	GAG Glu	CTG	ACA Thr	CCA
GAA Glu	CTG Leu 25	AGC Ser	GTC Val	GAG Glu
CCT	ATT Ile	AGC Ser 40	ACG Thr	TTT Phe
GTT Val	CTG Leu	GCC	CTC Leu 55	GTG Val
GAT Asp	TCC	TGT Cys	AGG Arg	GCT
GGA G1y 5	TTC	CTC	ACC Thr	GTC
AAG Lys	CGC Arg 20	TAC	66C 61y	GAG Glu
GAT Asp	GAG Glu	ATG Met 35	CCG Pro	CCC
ACT Thr	AAG Lys	TCT Ser	666 G1y 50	CCA
GTG Val	AAG Lys	ACA Thr	TTC Phe	TTC

2) INFORMATION FOR SEQ ID NO:8:

75

70

SEQUENCE CHARACTERISTICS:
(A) LENGTH: 78 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

Ser Arg Glu 15 Val Thr Asp Lys Gly Asp Val Pro Glu Gly Tyr Ser Val 1

Lys Lys Glu Arg Phe Ser Leu Ile Leu Glu Ser Ala Ser Thr Asn Gln 20

Ser Leu Asp Arg Gly Glu Gln Tyr 45 Ser 40 Ser Met Tyr Leu Cys Ala

Phe Gly Pro Gly Thr Arg Leu Thr Val Thr Glu Asp Leu Lys Asn Val 50

Phe Pro Pro Glu Val Ala Val Phe Glu Pro Ser Glu Ala Glu 65

(2) INFORMATION FOR SEQ ID NO:9:

i) SEQUENCE CHARACTERISTICS:

(A) LENGIH: 240 base pairs

TYPE: nucleic acid STRANDEDNESS: double

(C) STRANDEDNESS: doul (D) TOPOLOGY: unknown (ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:

(A) NAME/KEY: CDS

(B) LOCATION: 1..240

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

GTG ACT GAT AAG GGA GAT GTT CCT GAA GGG TAC AGT GTC TCT AGA GAG Val Thr Asp Lys Gly Asp Val Pro Glu Gly Tyr Ser Val Ser Arg Glu

Ala Ser Thr Asn TCC GCC AGC ACC AAC Lys Glu Arg Phe Ser Leu Ile Leu Glu Ser 20 GAG CIG ATT TTC TCC CTG AAG AAG ACA

CCA GGC ACC CGG CTG ACA GTG CTC GAG GAC CTG AAA Pro Gly Thr Arg Leu Thr Val Leu Glu Asp Leu Lys TAC CTC TGT GCC AGC AGT TTA ACC TCC GTC ACA GAT ACG Tyr Leu Cys Ala Ser Ser Leu Thr Ser Val Thr Asp Thr 40 GGC 6 TAT TTT (Tyr Phe (50 TCT ATG 1 Ser Met 1 35 CAG Gln

AAC GTG TTC CCA CCC GAG GTC GCT GTG TTT GAG CCA TCA GAA GCA GAG Asn Val Phe Pro Pro Glu Val Ala Val Phe Glu Pro Ser Glu Ala Glu 65

(2) INFORMATION FOR SEQ ID NO:10:

(A) LENGTH: 80 amino acids SEQUENCE CHARACTERISTICS: (i)

TYPE: amino acid TOPOLOGY: linear <u>(e)</u>

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

Val Thr Asp Lys Gly Asp Val Pro Glu Gly Tyr Ser Val Ser Arg Glu $_{\rm 1}$

Lys Lys Glu Arg Phe Ser Leu Ile Leu Glu Ser Ala Ser Thr Asn Gln

Ser Leu Thr Ser Val Thr Asp Thr Thr Ser Met Tyr Leu Cys Ala Ser 35 Arg Leu Thr Val Leu Glu Asp Leu Lys 9 Thr Tyr Phe Gly Pro Gly 50

Glu Asn Val Phe Pro Pro Glu Val Ala Val Phe Glu Pro Ser Glu Ala

(2) INFORMATION FOR SEQ ID NO:11:

SEQUENCE CHARACTERISTICS: (i)

LENGTH: 252 base pairs

TYPE: nucleic acid (E)

STRANDEDNESS: double TOPOLOGY: unknown

<u>(e</u>

(ii) MOLECULE TYPE: DNA (genomic)

FEATURE: (ix)

(A) NAME/KEY: CDS
(B) LOCATION: 1..252

SEQUENCE DESCRIPTION: SEQ ID NO:11: (xi)

GGG TAC AGC GTC TCT CGG GAG AAG Gly Tyr Ser Val Ser Arg Glu Lys CAG AAA GGA GAT ATA GCT GAA Gln Lys Gly Asp Ile Ala Glu 5 Phe

CCG ACA Pro Thr CAA AAG AAC Gln Lys Asn CCT CTC ACT GTG ACA TCG GCC Pro Leu Thr Val Thr Ser Ala Pro Leu Phe TTTTCC Glu Ser GAA

Gly CTA ACC Leu Thr 999 Gly AGT AGT ATT GGG GGA CAA Ser Ser Ile Gly Gly Gln Cys Ala Ser ဥ္ဌာ \mathtt{TGT} CIC Leu TTC TAT Phe Tyr

Len GAG Glu GTG Val AAA AAC GTG TTC CCA CCC GAG GTC GCT GTG TTT Lys Asn Val Phe Pro Pro Glu Val Ala Val Phe 70 CAG TAC TTC GGC GCG GGG ACC CGG CCC TCA Gln Tyr Phe Gly Ala Gly Thr Arg Pro Ser 55 Lys Asn 1 50 GAG GAC CTG AAA AAC

TCA GAA GCA GAG Ser Glu Ala Glu (2) INFORMATION FOR SEQ ID NO:12:

(A) LENGTH: 84 amino acids (B) TYPE: amino acid (D) TOPOLOGY: linear

SEQUENCE CHARACTERISTICS:

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

Ser Arg Glu Lys Phe Gln Lys Gly Asp Ile Ala Glu Gly Tyr Ser Val Lys Glu Ser Phe Pro Leu Thr Val Thr Ser Ala Gln Lys Asn Pro Thr

Ala Phe Tyr Leu Cys Ala Ser Ser Ile Gly Gly Gln Gly Leu Thr Gly 35 45

Ala Lys Asn Ile Gln Tyr Phe Gly Ala Gly Thr Arg Pro Ser Val Leu 50

Glu Asp Leu Lys Asn Val Phe Pro Pro Glu Val Ala Val Phe Glu Pro

Ser Glu Ala Glu

(2) INFORMATION FOR SEQ ID NO:13:

SEQUENCE CHARACTERISTICS: (Ţ)

LENGTH: 237 base pairs

TYPE: nucleic acid

STRANDEDNESS: double

TOPOLOGY: unknown **@** 0

MOLECULE TYPE: DNA (genomic) (ii)

FEATURE: (ix)

(A) NAME/KEY: CDS

LOCATION: 1..237

SEQUENCE DESCRIPTION: SEQ ID NO:13: (xi)

Glu CAG $\frac{Lys}{15}$ CGA AAA Arg CCC AAC GAA GGG TAC AAA GTC TCT Glu Gly Tyr Lys Val Ser GGA GAT ATA GCT GAA Gly Asp Ile Ala Glu 5 AAA Gln Lys CAG Phe

ACC Pro Asn TCG CCC AGC Ser Pro Ser Glu Ser TTC CCC CTG ATC CTG GAG Len Ile Pro Leu Phe AGG AAT Arg Asn AAG Lys

GAG Tyr Asn GGA ACC TAC AAT Thr Gly GCC AGC AGT TTG GGG Gly Leu Ser Ser Ala \mathtt{TGT} Cys Phe TTC Tyr 35 TAC CTGLen TCT Ser

Asn AAA Leu Glu Asp Leu Lys GAG GAC CTG CTA GTG Val Thr CTC ACC Leu SSS Gly Thr Arg ACA GGG Pro CCA 999 Gly Phe TTC

Val Phe Pro Pro Glu Val Ala Val Phe Glu Pro Ser Glu Ala Glu 65 GTG TTC CCA CCC GAG GTC GCT GTG TTT GAG CCA TCA GAA GCA

(2) INFORMATION FOR SEQ ID NO:14:

SEQUENCE CHARACTERISTICS: (A) LENGTH: 79 amino acids (i)

TYPE: amino acid <u>(e)</u> (e)

TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

Phe Gln Lys Gly Asp Ile Ala Glu Gly Tyr Lys Val Ser Arg Lys Glu $_{\rm 1}$

Lys Arg Asn Phe Pro Leu Ile Leu Glu Ser Pro Ser Pro Asn Gln Thr

Ser Leu Tyr Phe Cys Ala Ser Ser Leu Gly Gly Thr Tyr Asn Glu Gln 35

Phe Phe Gly Pro Gly Thr Arg Leu Thr Val Leu Glu Asp Leu Lys Asn 50 60

Val Phe Pro Pro Glu Val Ala Val Phe Glu Pro Ser Glu Ala Glu

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:

LENGTH: 342 base pairs (A)

TYPE: nucleic acid

STRANDEDNESS: double

TOPOLOGY: unknown (Ú) (£) (ii) MOLECULE TYPE: DNA (genomic)

FEATURE: (ix)

NAME/KEY: CDS (A)

LOCATION: 1..342

SEQ ID NO:15 SEQUENCE DESCRIPTION: (xi)

Trp 15 TIC Phe CIC Leu GGG GCA ACA CCT TAT Gly Ala Thr Pro Tyr AGG TGC AAC TAT TCC TAT Arg Cys Asn Tyr Ser Tyr Leu

Ser TTT TAC CCC CTG AAG Pro Leu Lys CAG CTG (Glu In Inch CIC Leu 66C 61y CAA Gln 66c 61y CCC \mathbb{T}^{CC} Ser CAG GTC

Glu GAA Glu Ala GAG GCT $\mathbf{T}\mathbf{T}\mathbf{I}$ Gly ၁၅၅ CAA GGC ATT AAA Gln Gly Ile Lys GTT Val Leu CTG Thr ACT Asp GGA Gly

AGT Ser CAT TGG GTGVal $\mathbf{T}^{\mathbf{C}\mathbf{I}}$ Ser Pro AGG AAA CCC Lys Arg CTG Leu TTC AAT Phe Asn TCC Ser \mathbf{I} C \mathbf{I} Ser CAA Gln AGT Ser Arg AGC Ser TAC Ser GCT GAT TCA Asp GGT Gly GCT GTG Ala Val TGT GCT cys TTC Phe Tyr TAC GAG Glu Ala GCT Ala Asp 65 GAT Pro Asp CCA CTA GTC TCT Leu Val Ser ACT ATG CTT Thr Met Leu GGG Lys AAG 666 G1y Phe Thr Leu 82

AGT Ser TCC Ser Gln Leu Arg Asp Ser 105 CTG AGA GAC TCT CCT GCC GTG TAC Pro Ala Val Tyr Gln Asn Pro Asp 100 GAC CAG AAC CCT

GAC AAG

Asp Lys

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 114 amino acids

TYPE: amino acid

TOPOLOGY: linear (<u>a</u>) (ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

Leu Arg Cys Asn Tyr Ser Tyr Gly Ala Thr Pro Tyr Leu Phe Trp

Val Gln Ser Pro Gly Gln Gly Leu Gln Leu Pro Leu Lys Tyr Phe Ser

Gly Asp Thr Leu Val Gln Gly Ile Lys Gly Phe Glu Ala Glu Phe Lys 35

Ser Phe Asn Leu Arg Lys Pro Ser Val His Trp Ser Gln Arg Ser 50

Asp Ala Ala Glu Tyr Phe Cys Ala Val Gly Ala Asp Ser Gly Tyr 65

CAG

Ser

Phe

Ile Gly Lys Gly Thr Met Leu Leu Val Ser Pro Asp 85 Thr Leu Thr Phe

Ser Ser $\frac{\text{Lys}}{110}$ Ser Gln Leu Arg Asp 105 Tyr Asp Pro Ala Val 100 Gln Asn Pro

Asp Lys

(2) INFORMATION FOR SEQ ID NO:17

SEQUENCE CHARACTERISTICS: (i)

LENGTH: 336 base pairs

TYPE: nucleic acid

STRANDEDNESS: double TOPOLOGY: unknown MOLECULE TYPE: DNA (genomic)

FEATURE: (ix)

(A) NAME/KEY: CDS (B) LOCATION: 1...

SEQUENCE DESCRIPTION: SEQ ID NO:17 (xi)

Tyr TTC TGG TAT Trp 15 TTTTAC Tyr 30 Phe CTC CAG CTG CTC CTG AAG Leu Gln Leu Leu Lys Leu TAT GGG GCA ACA CCT TAT Tyr Gly Ala Thr Pro Tyr CCC GGC CAA GGC CTC CAG CTG CTC Pro Gly Gln Gly Leu Gln Leu TAT TCC TYY Ser TGC AAC Cys Asn TCC CAG Gln CTG AGG Leu Arg GTC

GGA GAC ACT CTG GTT CAA GGC ATT AAA GGC TTT GAG GCT GAA TTT AAG

Ser

Val

Lys	AGT Ser	ATG Met 80	AAC Asn	AAG Lys
Phe	TGG Trp	GAC Asp	CAG Gln 95	GAC Asp
Glu	CAT His	AAT Asn	ATC Ile	AGT Ser 110
Ala 45	GTG Val	CAC His	AAT Asn	TCC
Glu	TCT Ser 60	ACC Thr	CCA Pro	AAA Lys
Phe	CCC	CCC Pro 75	AAA Lys	TCT Ser
Gly	AAA Lys	GGT Gly	GTA Val 90	GAC
Lys	AGG Arg	GTG Val	ACA	AGA Arg 105
I.1e 40	CTG	GCT	CTG	CTG
ı Gly	AAT Asn 55	TGT Cys	AGA Arg	CAG Gln
Gln	TTC Phe	TTC Phe 70	ACC Thr	TAC TYF
Val	TCC	TAC Tyr	GGG G1y 85	GTG Val
Leu	TCT	GAG Glu	GCA Ala	GCC Ala 100
Thr 35	CAA Gln	GCT	GGA Gly	CCT
Asp	AGT Ser 50	GCT	TTT Phe	GAC Asp
Gly	AGG Arg	GAT Asp 65	CGC Arg	CCT

(A) LENGTH: 112 amino (B) TYPE: amino acid (D) TOPOLOGY: linear (ii) MOLECULE TYPE: protein

SEQUENCE CHARACTERISTICS: (A) LENGTH: 112 amino acids

(i)

(2) INFORMATION FOR SEQ ID NO:18:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

Leu Arg.Cys Asn Tyr Ser Tyr Gly Ala Thr Pro Tyr Leu Phe Trp

Ser Ser Pro Gly Gln Gly Leu Gln Leu Leu Leu Lys Tyr Phe

Thr Leu Val Gln Gly Ile Lys Gly Phe Glu Ala Glu Phe Lys Gly Asp

Phe Asn Leu Arg Lys Pro Ser Val His Trp Gln Ser Ser

Arg Ser

Asp Ala Ala Glu Tyr Phe Cys Ala Val Gly Pro Thr His Asn Asp Met 65

Arg Phe Gly Ala Gly Thr Arg Leu Thr Val Lys Pro Asn Ile Gln Asn 85

Asp Lys Ser 110 Pro Asp Pro Ala Val Tyr Gln Leu Arg Asp Ser Lys Ser 100

(2) INFORMATION FOR SEQ ID NO:19:

LENGTH: 324 base pairs SEQUENCE CHARACTERISTICS:

TYPE: nucleic acid € (E) (E)

STRANDEDNESS: double

TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:

LOCATION: 1..324 NAME/KEY: CDS

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

Trp 15 Gly Ser Pro Glu Leu Phe GAA CTC GGG AGT CCT TAT TCT Tyr Ser Tyr Ser 5 AGG TGC AAC TAT TCC Cys Asn Leu

Ser ATC Ile CAA CGC CTC CAG TTA CTC TTG AGA CAC Gln Arg Leu Gln Leu Leu Leu Arg His 25 AGA Arg TCC TAC Tyr CAG Gln

GAG ACA Glu Thr 66C 61y ACT GCT GAC CTT AAC AAA Thr Ala Asp Leu Asn Lys 40 299 Lys Gly ATC AAA Ile Lys GAG AGC 1 Glu Ser

GAC TCA GCC ATG Ser CAA GAG GAA GAC Gln Glu Glu Asp TTT GCT (Pro CCA TTC CAC CTG AAG AAA Phe His Leu Lys Lys

TCT Ser

Leu TTA CTT TTG Leu Leu ACA AGC 1 Thr Ser 1 CTG CAG GCA Leu Gln Ala TAC TGT GCT CTA GCG Tyr Cys Ala Leu Ala

TAT Tyr 65

Asn Pro Asp Pro CTA GTT AAA CCA AAT ATC CAG AAC CCT GAC CCT Leu Val Lys Pro Asn Ile Gln Asn Pro Asp Pro Leu 85 Val GTGAGG Arg GAA

AAG Lys AGT GAC $\mathbf{I}^{\mathbf{CC}}$ AAA

Asp Ser Ser Lys TCT AGA GAC Arg Asp CAG Civ T Gln Leu Ar

Val

INFORMATION FOR SEQ ID NO:20:

SEQUENCE CHARACTERISTICS: (<u>;</u>

LENGTH: 108 amino acids (B)

TYPE: amino acid

TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

Leu Arg Cys Asn Tyr Ser Tyr Ser Gly Ser Pro Glu Leu Phe Trp 1

Ser Ile Ser Arg Gln Arg Leu Gln Leu Leu Leu Arg His 20

Ile Lys Gly Phe Thr Ala Asp Leu Asn Lys Gly Glu 45

Arg Glu Ser

Val Gln Tyr

Ser Phe His Leu Lys Lys Pro Phe Ala Gln Glu Glu Asp Ser Ala Met

Tyr Tyr Cys Ala Leu Ala Leu Gln Ala Thr Ser Leu Leu Glu Glu Glu 65 . 75 Ile Gln Asn Pro Asp Pro Ala Glu Pro Arg Val Leu Val Lys Pro Asn

Ser Asp Lys Val Tyr Gln Leu Arg Asp Ser Lys Ser 100

INFORMATION FOR SEQ ID NO:21 (2) SEQUENCE CHARACTERISTICS (i,

LENGTH: 352 base pair

TYPE: nucleic acid (B)

STRANDEDNESS: double TOPOLOGY: unknown MOLECULE TYPE: DNA (genomic)

FEATURE: (ix)

(A) NAME/KEY: CDS (B) LOCATION: 2...

LOCATION: 2..352

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

Ser Val Tyr TTG TCA CTG GAT TTA GAG TCT CTC AGC TGC AGT Leu Ser Leu Asp Leu Glu Ser Leu Ser Cys Ser

ACA GTC AGC

TAC

Gly Lys Gly Pro Pro Gln Asp Trp Tyr Arg

TGG TAT AGG CAA GAT CCT GGG AAA

CTG TTC

GGT TTA AGA GGG

O

Leu Arg Gly Leu Phe

Gly

GAA AAA g]n GAG Lys GAA AAG Glu Glu GGG GAA G1yAla GCT TCA Ser TAT Leu Tyr CIGACC

Phe Leu Phe

 $\mathbf{T}\mathbf{L}\mathbf{C}$

CIC

Glu

Ser Glu Lys Lys (Leu Thr

Thr ACA

Lys

Leu

Arg AGG

ပ္ပင္သ Ala

Ile

Leu

Phe

CTG CAC His

TTT

AGC

GAA

AAG

ACA AAG

TGT GCT GCG AGG CGA GCC ACT TAT CTC CCT AAA CCT GAA GAC TCA ပ္ပင္သ Ala Pro Lys Pro Glu Asp Ser Ala Thr Tyr Leu Cys Ala Ala Arg Arg 65

Val GGA ACC ATG TTA AAG Gly Thr Met Leu Lys 90 GGC CAG AAG CTG CTC TTT GCA AGG Gly Gln Lys Leu Leu Phe Ala Arg TCA GAT G Ser Asp G 80

CAG CTG GTG TAC GCC (Ala CAG AAC CCT GAC CCT Gln Asn Pro Asp Pro CCT GAC CCI AAC CTT AAT Leu Asn Asp GAT

Asp Lys GAC AAG TCT AAA TCC AGT G Ser Lys Ser Ser A (2) INFORMATION FOR SEQ ID NO:22:

(A) LENGTH: 117 amino acids (B) TYPE: amino acid (D) TOPOLOGY: linear SEQUENCE CHARACTERISTICS:

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

Leu Ser Leu Asp Leu Glu Ser Leu Ser Cys Ser Tyr Thr Val Ser 1

Leu Arg Gly Leu Phe Trp Tyr Arg Gln Asp Pro Gly Lys Gly Pro Glu $20\,$

Phe Leu Phe Thr Leu Tyr Ser Ala Gly Glu Glu Lys Glu Lys Glu Arg

Leu Lys Ala Thr Leu Thr Lys Lys Glu Ser Phe Leu His Ile Thr 50

Pro Lys Pro Glu Asp Ser Ala Thr Tyr Leu Cys Ala Ala Arg Arg 65 75

Asp Asp Gly Gln Lys Leu Leu Phe Ala Arg Gly Thr Met Leu Lys Val

Gln Asn Pro Asp Pro Ala Val Tyr Gln Leu Arg Asp Ser 100 Ile Leu Asn

Ser Asp Lys Ser Lys

(2) INFORMATION FOR SEQ ID NO:23:

SEQUENCE CHARACTERISTICS:

LENGTH: 352 base pairs TYPE: nucleic acid

STRANDEDNESS: double TOPOLOGY: unknown

MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:

(A) NAME/KEY: CDS (B) LOCATION: 2...

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

TGC AGT TAC ACA GTC Tyr Thr Val Cys Ser CTC AGC Leu Ser Leu Asp Leu Glu Ser Leu Ser TTA GAG TCT TTG TCA CTG GAT ပ

15	CCT	GAA Glu	ACA Thr	CGA Arg	GTG Val 95	GAC Asp
-	66C 61y 30	AAA Lys	ATC Ile	AGG Arg	AAG Lys	AGA Arg 110
	AAA Lys	GAG Glu 45	CAC His	GTG Val	TTA Leu	CTG Leu
	GGG Gly	AAG Lys	CTG Leu 60	GCT Ala	ATG Met	CAG Gln
	CCT (GAA	TTT Phe	TGT Cys 75	ACC Thr	TAC TYr
	GAT	GAA	AGC Ser	CTC	GGA G1y 90	GTG Val
10	CAA Gln 25	666 G1y	GAA Glu	TAT Tyr	AGG Arg	GCC Ala 105
	AGG Arg			ACT	GCA Ala	CCT
	TAT	TCA	AAG Lys 55	GCC Ala	TTT Phe	GAC Asp
	TGG T	TAT TYr	ACA Thr	TCA Ser 70	CTC	CCT Pro
ເດ	TTC Phe CTG Leu		TTA	GAC Asp	CTG Leu 85	AAC Asn
	CTG Leu 20 20 ACC Thr		ACA Thr	GAA Glu	AAG Lys	CAG Gln 100
,	GGG	TTC Phe 35	GCC	CCT	CAG Gln	ATC
H	AGA Arg	CTC	AAA Lys 50	AAA Lys	66C 61y	AAT
	TTA			CCT Pro 65	GAT Asp	CTT Leu
	GGT	GAA Glu	AGG Arg	GCC	TCA Ser 80	GAT Asp

TCT AAA TCC AGT GAC AAG
Ser Lys Ser Ser Asp Lys
115
(2) INFORMATION FOR SEQ ID NO:24:

(i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 117 amino acids (B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

Leu Ser Leu Asp Leu Glu Ser Leu Ser Cys Ser Tyr Thr Val 1

Phe Leu Phe Thr Leu Tyr Ser Ala Gly Glu Glu Lys Glu Lys Glu Arg 35

Leu Lys Ala Thr Leu Thr Lys Lys Glu Ser Phe Leu His Ile Thr Ala 50 60

Pro Lys Pro Glu Asp Ser Ala Thr Tyr Leu Cys Ala Val Arg Arg 65

Asp Asp Gly Gln Lys Leu Leu Phe Ala Arg Gly Thr Met Leu Lys Val 85 Leu Asn Ile Gln Asn Pro Asp Pro Ala Val Tyr Gln Leu Arg Asp Ser 100

Lys Ser Ser Asp Lys 115

(2) INFORMATION FOR SEQ ID NO:25:

SEQUENCE CHARACTERISTICS:

(A) LENGTH: 352 base pairs(B) TYPE: nucleic acid

STRANDEDNESS: double TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

FEATURE: (ix)

(A) NAME/KEY: CDS (B) LOCATION: 2...

LOCATION: 2..352

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

TTG TCA CTG GAT TTA GAG TCT CTC AGC TGC AGT TAC ACA GTC AGC Leu Ser Leu Ser Leu Ser Tyr Thr Val Ser 1 5 U

GGC CCT Gly Pro Lys CAA GAT CCT GGG AAA GIn Asp Pro Gly Lys TAT AGG (Tyr Arg (CTG TTC TGG Leu Phe Trp GGG TTA AGA Leu Arg GGT

Glu GAG AAA Glu Lys GAA AAG Glu GGG GAA (G1y Glu (Ala TCA GCT Ser ACC CTG TAT Thr Leu Tyr Phe CTC TTC Leu TTCPhe GAA Glu

CTG CAC ATC Leu His Ile . ANG AAG GAA AGC TTT C. Tys Lys Glu Ser Phe 1. TTA ACA AAG AAG ACA TTA ACA Thr Leu Thr Ala ပ္ပပ္သ Lys 50 Arg Leu AGG

c TGT GCT GTG AGG C 1 Cys Ala Val Arg A1 75 GAC TCA GCC ACT TAT CTC TGT Asp Ser Ala Thr Tyr Leu Cys Pro Lys Pro Glu Asp GAA CCT GCC CCT AAA

GTG

GGC CAG AAG CTG CTC TTT GCA AGG GGA ACC ATG TTA AAG Gly Gln Lys Leu Leu Phe Ala Arg Gly Thr Met Leu Lys 85 GAT CTT AAT ATC CAG AAC CCT GAC CCT GCC GTG TAC CAG CTG AGA ASP Leu Asn Ile Gln Asn Pro Asp Pro Ala Val Tyr Gln Leu Arg 100 GAC AAG Ser Asp Lys AAA TCC AGT Ser Ser Asp TCT Ser

(2) INFORMATION FOR SEQ ID NO:26:

SEQUENCE CHARACTERISTICS:
(A) LENGTH: 117 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear (i)

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

Gly Ser Leu Ser Leu Asp Leu Glu Ser Leu Ser Cys Ser Tyr Thr Val 1

Leu Arg Gly Leu Phe Trp Tyr Arg Gln Asp Pro Gly Lys Gly Pro Glu $20\,$

Phe Leu Phe Thr Leu Tyr Ser Ala Gly Glu Glu Lys Glu Lys Glu Arg 35

Leu Lys Ala Thr Leu Thr Lys Lys Glu Ser Phe Leu His Ile Thr Ala 50 60

Asp Gly Gin Lys Leu Leu Phe Ala Arg Gly Thr Met Leu Lys Val Asp 95 Ser Ser Leu Asn Ile Gln Asn Pro Asp Pro Ala Val Tyr Gln Leu Arg Asp Pro Lys Pro Glu Asp Ser Ala Thr Tyr Leu Cys Ala Val Arg Arg 65

105

Ser Asp Lys Lys Ser

(2) INFORMATION FOR SEQ ID NO:27:

SEQUENCE CHARACTERISTICS:

(A) LENGTH: 352 base pairs(B) TYPE: nucleic acid STRANDEDNESS: double

TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

FEATURE: (ix)

(A) NAME/KEY: CDS

(B) LOCATION: 2..352

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

C TTG TCA CTG GAT TTA GAG TCT CTC AGC TGC AGT TAC ACA GTC AGC Leu Ser Leu Ser Leu Ser Tyr Thr Val Ser 15

GGT TTA AGA TGG CTG TTC TGG TAT AGG CAA GAT CCT GGG AAA GGC CCT

GAG AAA (Leu Phe Trp Tyr Arg Gln Asp Pro Gly Lys Gly 20 GAA AAG (Glu Lys (GAA G GGG GCT Ser TCA TAT Tyr CTG Phe Ala Leu ည္ပ TTC Trp Arg Glu Phe Leu Leu TTC GAA

Thr Ile ATC TTT CTG CAC. CTA AAA GCC ACA TTA ACA AAG AAG GAA AGC Leu Lys Ala Thr Leu Thr Lys Lys Glu Ser 50 AGG

Arg Val GTG Ala GCT TGT (Cys 75 CIC Tyr Leu TAT ACT ' TCA GCC Ser Ala CCT AAA CCT GAA GAC Pro Lys Pro Glu Asp CCC

Val AAG GGA ACC ATG TTA A GIY Thr Met Leu I 90 AAG CTG CTC TTT GCA AGG Lys Leu Leu Phe Ala Arg 85 CAG Gln ၁၅၅ Gly GAT Asp

Ser

TCA

CTG Gln Leu CAG TAC Tyr CAG AAC CCT GAC CCT GCC GTG Gln Asn Pro Asp Pro Ala Val 100 CTT AAT ATC Asn

GAC AAG Asp Ser 115 $\mathbf{I}^{\mathbf{CC}}$ Ser Γ CT Ser

(2) INFORMATION FOR SEQ ID NO:28:

SEQUENCE CHARACTERISTICS:

(A) LENGTH: 117 amino acids (B) TYPE: amino acid (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

Leu Ser Leu Asp Leu Glu Ser Leu Ser Cys Ser Tyr Thr Val Ser 1

Leu Arg Trp Leu Phe Trp Tyr Arg Gln Asp Pro Gly Lys Gly Pro Glu 20

Phe Leu Phe Ala Leu Tyr Ser Ala Gly Glu Glu Lys Glu Lys Glu Arg 35

Leu Lys Ala Thr Leu Thr Lys Lys Glu Ser Phe Leu His Ile Thr Ala 50 60

Pro Lys Pro Glu Asp Ser Ala Thr Tyr Leu Cys Ala Val Arg Arg 65

Asp Asp Gly Gln Lys Leu Leu Phe Ala Arg Gly Thr Met Leu Lys Val 85

Leu Asn Ile Gln Asn Pro Asp Pro Ala Val Tyr Gln Leu Arg Asp Ser 100

Ser Asp Lys 115 Lys Ser

(2) INFORMATION FOR SEQ ID NO:29:

SEQUENCE CHARACTERISTICS:

⁽A) LENGTH: 343 base pairs(B) TYPE: nucleic acid(C) STRANDEDNESS: double

(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:

(A) NAME/KEY: (B) LOCATION:

LOCATION: 2.343

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:

GAT GTC TCC ATG AAC TGC ACT TCT TCA AGC ATA TTT AAC ACC TGG Asp Val Ser Met Asn Cys Thr Ser Ser Ser Ile Phe Asn Thr Trp 1

Ø

GGT CCT GTC CTC TTG Gly Pro Val Leu Leu 25 CAG GAC CCT GGG GAA Gln Asp Pro Gly Glu TGG TAC AAG Trp Tyr Lys CTA

CAG Gln Thr ACT Len GGA AGA (Gly Arg] ACC TCA AAT Thr Ser Asn GAA TTG Glu Leu GGT Ala 35 Lys TyrTAT

TTA

 $ext{TLL}$

GCA CTG AAT ATC Leu Asn Ile TTC AGC GAC AGA AAG gg

ATA Ile

Ser

Ser Ser Asp Arg Lys ACC Ile Gly

cTcLeu GCC Gln 7 Gly Ala GCT Cys ATC TAC Tiv / Ile Tyr Phe C 70 ၁၅၅ Gly GTA Val GAT Asp Ser CCT AGT Pro

Ile GTC Thr ACG TIG ren ' Ser ACA AGT Thr 999 G1yACA Thr TTT Phe TAT Tyr CAG AAC

Ile Gln Asn Pro Asp Pro Ala Val Tyr Gln Leu Arg Asp Ser Lys

AGT GAC AAG

Ser Asp Lys

(2) INFORMATION FOR SEQ ID NO:30:

(i) SEQUENCE CHARACTERISTICS:

A) LENGIH: 114 amino acids

(B) TYPE: amino acid(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:

Trp Leu 15 Asp Val Ser Met Asn Cys Thr Ser Ser Ser Ile Phe Asn Thr 1 Tyr Lys Gln Asp Pro Gly Glu Gly Pro Val Leu Leu Ile Ala Leu 20 Trp

Tyr Lys Ala Gly Glu Leu Thr Ser Asn Gly Arg Leu Thr Ala Gln Phe

Gly Ile Thr Arg Lys Asp Ser Phe Leu Asn Ile Ser Ala Ser Ile Pro

Ser Asp Val Gly Ile Tyr Phe Cys Ala Gly Gln Ala Leu Thr Gly Asn 65

Gln Phe Tyr Phe Gly Thr Gly Thr Ser Leu Thr Val Ile Pro Asn Ile 85

Ser Ser Lys 110 Pro Asp Pro Ala Val Tyr Gln Leu Arg Asp Ser 100 Gln Asn

Asp Lys

(2) INFORMATION FOR SEQ ID NO:31:

SEQUENCE CHARACTERISTICS:

LENGTH: 408 base pairs

TYPE: nucleic acid

STRANDEDNESS: double TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

FEATURE: (ix)

(A) NAME/KEY: CDS

(B) LOCATION: 1..408

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:31:

CTT GTC ACT GGA TTT AGA GTC TCT CAG CTG GTG GAG CAG AGC CCT CAA Leu Val Thr Gly Phe Arg Val Ser Gln Leu Val Glu Gln Ser Pro Gln 1

TGT GCT TAT Cys Ala Tyr 30 TTG ATA GTC CAG AAA GGA GGG ATT TCA ATT ATA AAC Leu Ile Val Gln Lys Gly Gly Ile Ser Ile Ile Asn TCI Ser

GGG G1y GAG AAC ACT GCG TTT GAC TAC TTT CCA TGG TAC CAA CAA TTC CCT Glu Asn Thr Ala Phe Asp Tyr Phe Pro Trp Tyr Gln Gln Phe Pro 35

AAG Lys	TTC Phe 80	TTC	TTA	CTG
GAA Glu	CAG Gln	TAC TYr 95	GAG Glu	CAG Gln
AGT Ser	AAG Lys	ACC Thr	ACG Thr 110	TAC
GTG Val	GCC Ala	GCC	GGA Gly	GTG Val 125
GAT Asp 60	AGT	TCA	CAG Gln	GCC
CCA	AAA Lys 75	gac Asp	GGA Gly	CCT
CGT	AAT Asn	66A 61y 90	TTC Phe	GAC
ATA Ile	TTC Phe	CCT	ATC Ile 105	CCT
GCC	Tcc Ser	CAG Gln	CTT Leu	AAC Asn 120
ATA Ile 55	ATC Ile	TCC Ser	aag Lys	CAG Gln
TTG	ACA Thr 70	GAT Asp	GGA G1y	ATC Ile
TTA	TTC	ATG Met 85	GGA Gly	AAT Asn
GCA	AGA Arg	ATC Ile	GAG Glu 100	CCC
CCT	GGA Gly	CAT	GCA	AAA Lys 115
66C 61Y 50	GAA Glu	TTG	GCA	GTG Val
AAA Lys	AAA Lys 65	TCA	TGT Cys	TCT Ser

(2) INFORMATION FOR SEQ ID NO:32:

AGA GAC TCT AAA TCC AGT GAC AAG Arg Asp Ser Lys Ser Ser Asp Lys 130

SEQUENCE CHARACTERISTICS:
(A) LENGTH: 136 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear (i,

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:32:

Leu Val Thr Gly Phe Arg Val Ser Gln Leu Val Glu Gln Ser Pro Gln 1 15

Ser Leu Ile Val Gln Lys Gly Gly Ile Ser Ile Ile Asn Cys Ala Tyr 20 30

Glu Asn Thr Ala Phe Asp Tyr Phe Pro Trp Tyr Gln Gln Phe Pro

5 45 45 6 Ale I.eu I.e Ale I.e Ard Pro Asp Val Ser Glu Lys

Lys Gly Pro Ala Leu Leu Ile Ala Ile Arg Pro Asp Val Ser Glu Lys 50 50

Lys Glu Gly Arg Phe Thr Ile Ser Phe Asn Lys Ser Ala Lys Gln Phe 65

Gln Pro Gly Asp Ser Ala Thr Tyr 90 Ser Leu His Ile Met Asp Ser

Glu Leu Thr Glu Gly Gly Lys Leu Ile Phe Gly Gln Gly 100 Cys Ala Ala

Ser Val Lys Pro Asn Ile Gln Asn Pro Asp Pro Ala Val Tyr Gln Leu 115

Arg Asp Ser Lys Ser Ser Asp Lys 130 (2) INFORMATION FOR SEQ ID NO:33:

i) SEQUENCE CHARACTERISTICS:(A) LENGTH: 240 base pairs

(B) TYPE: nucleic acid

STRANDEDNESS: double TOPOLOGY: unknown DNA (genomic) MOLECULE TYPE: (ii)

FEATURE: (ix)

(A) NAME/KEY: (B) LOCATION:

LOCATION: 1..240

SEQUENCE DESCRIPTION: SEQ ID NO:33: (xi) GAG Glu AAA Lys 15 GTC TCT CGA Val Ser Arg Arg TAC AAA (Tyr Lys GAA GGG 7 Glu Gly 7 GCT (ATA Ile GAT ASP GGA Lys AAA Gln Phe

ACC Thr CCC AAC CAG Pro Asn Gln AGC Ser CCC TCG GAG Glu ATC CTG (Ile Leu (CCC CTG Pro Leu TTC Asn AAT Arg AGG AAG Lys

Gly TAT Tyr TCC Ala CGA Arg TCT Ser TTC Pro ဗ္ဗင္ဗ AGT Ser 40 AGC ၁၁၅ Ala TGT TTC Tyr 35 TAC Len Ser

Lys AAA CTGLeu GAC Asp GAG Glu GTA Val 9 GTT Val ACC Leu TTA AGG Arg AAC Asn 55 Gly 999 TCG Ser Gly GGTPhe Thr ACC TAC Tyr

GCA Ala GAA Glu TCA Ser Glu Pro GAG CCA Phe $\mathbf{T}\mathbf{T}\mathbf{I}$ GTGVal GCT GTC Glu GAG Pro သည Pro S.C.A Phe GTG Asn Val AAC

INFORMATION FOR SEQ ID NO:34: (2)

SEQUENCE CHARACTERISTICS: (i)

LENGTH: 80 amino acids

TYPE: amino acid TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:34:

Phe Gln Lys Gly Asp Ile Ala Glu Gly Tyr Lys Val Ser Arg Lys Glu 10

Lys Arg Asn Phe Pro Leu Ile Leu Glu Ser Pro Ser Pro Asn Gln Thr

Ser Leu Tyr Phe Cys Ala Ser Ser Pro Phe Ser Arg Ala Ser Tyr Gly 35

Tyr Thr Phe Gly Ser Gly Asn Arg Leu Thr Val Val Glu Asp Leu Lys 50 60

Asn Val Phe Pro Pro Glu Val Ala Val Phe Glu Pro Ser Glu Ala Glu

- (2) INFORMATION FOR SEQ ID NO:35:
 - (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 18 base pairs
 - (B) TYPE: nucleic acid
 - (D) TOPOLOGY: unknown
 - (ii) MOLECULE TYPE: DNA (genomic)
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:35: CTGAGGTGCA ACTACTCA
- (2) INFORMATION FOR SEQ ID NO:36:
 - (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 24 base pairs
 - (B) TYPE: nucleic acid
 - (D) TOPOLOGY: unknown
 - (ii) MOLECULE TYPE: DNA (genomic)
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:36: GTGTTCCCAG AGGGAGCCAT TGCC
- (2) INFORMATION FOR SEQ ID NO:37:
 - (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 21 base pairs
 - (B) TYPE: nucleic acid
 - (D) TOPOLOGY: unknown
 - (ii) MOLECULE TYPE: DNA (genomic)
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:37:

GGTGAACAGT CAACAGGGAG A

- (2) INFORMATION FOR SEQ ID NO:38:
 - (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 21 base pairs
 - (B) TYPE: nucleic acid
 - (D) TOPOLOGY: unknown
 - (ii) MOLECULE TYPE: DNA (genomic)
 - (xi) SEQUENCE DESCRIPTION: SEQ ID NO:38:

ACAAGCATTA CTGTACTCCT A

(2) INFORMATION FOR SEQ ID NO:39:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 18 base pairs
 - (B) TYPE: nucleic acid
 - (D) TOPOLOGY: unknown
- (ii) MOLECULE TYPE: DNA (genomic)
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:39:

GGCCCTGAAC ATTCAGGA

- (2) INFORMATION FOR SEQ ID NO:40:
 - (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 20 base pairs
 - (B) TYPE: nucleic acid
 - (D) TOPOLOGY: unknown
 - (ii) MOLECULE TYPE: DNA (genomic)
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:40: GTCACTTTCT AGCCTGCTGA
- (2) INFORMATION FOR SEQ ID NO:41:
 - (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 21 base pairs
 - (B) TYPE: nucleic acid
 - (D) TOPOLOGY: unknown
 - (ii) MOLECULE TYPE: DNA (genomic)
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:41:
- (2) INFORMATION FOR SEQ ID NO:42:
 - (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 22 base pairs
 - (B) TYPE: nucleic acid
 - (D) TOPOLOGY: unknown
 - (ii) MOLECULE TYPE: DNA (genomic)
 - (xi) SEQUENCE DESCRIPTION: SEQ ID NO:42:

GGAGAGAATG TGGAGCAGCA TC

- (2) INFORMATION FOR SEQ ID NO:43:
 - (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 21 base pairs

- (B) TYPE: nucleic acid
- (D) TOPOLOGY: unknown
- (ii) MOLECULE TYPE: DNA (genomic)
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:43:

ATCTCAGTGC TTGTGATAAT A

- (2) INFORMATION FOR SEQ ID NO:44:
 - (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 24 base pairs
 - (B) TYPE: nucleic acid
 - (D) TOPOLOGY: unknown
 - (ii) MOLECULE TYPE: DNA (genomic)
 - (xi) SEQUENCE DESCRIPTION: SEQ ID NO:44:

ACCCAGCTGG TGGAGCAGAG CCCT

- (2) INFORMATION FOR SEQ ID NO:45:
 - (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 21 base pairs
 - (B) TYPE: nucleic acid
 - (D) TOPOLOGY: unknown
 - (ii) MOLECULE TYPE: DNA (genomic)
 - (xi) SEQUENCE DESCRIPTION: SEQ ID NO:45:

AGAAAGCAAG GACCAAGTGT T

- (2) INFORMATION FOR SEQ ID NO:46:
 - (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 24 base pairs
 - (B) TYPE: nucleic acid
 - (D) TOPOLOGY: unknown
 - (ii) MOLECULE TYPE: DNA (genomic)
 - (xi) SEQUENCE DESCRIPTION: SEQ ID NO:46:

CAGAAGGTAA CTCAAGCGCA GACT

- (2) INFORMATION FOR SEQ ID NO:47:
 - (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 19 base pairs
 - (B) TYPE: nucleic acid
 - (D) TOPOLOGY: unknown

- (ii) MOLECULE TYPE: DNA (genomic)
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:47: GCTTATGAGA ACACTGCGT
- (2) INFORMATION FOR SEQ ID NO:48:
 - (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 20 base pairs
 - (B) TYPE: nucleic acid
 - (D) TOPOLOGY: unknown
 - (ii) MOLECULE TYPE: DNA (genomic)
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:48:
 GCAGCTTCCC TTCCAGCAAT
- (2) INFORMATION FOR SEQ ID NO:49:
 - (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 20 base pairs
 - (B) TYPE: nucleic acid
 - (D) TOPOLOGY: unknown
 - (ii) MOLECULE TYPE: DNA (genomic)
 - (xi) SEQUENCE DESCRIPTION: SEQ ID NO:49:

AGAACCTGAC TGCCCAGGAA

- (2) INFORMATION FOR SEQ ID NO:50:
 - (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 21 base pairs
 - (B) TYPE: nucleic acid
 - (D) TOPOLOGY: unknown
 - (ii) MOLECULE TYPE: DNA (genomic)
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:50: CATCTCCATG GACTCATATG A
- (2) INFORMATION FOR SEQ ID NO:51:
 - (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 19 base pairs
 - (B) TYPE: nucleic acid
 - (D) TOPOLOGY: unknown
 - (ii) MOLECULE TYPE: DNA (genomic)
 - (xi) SEQUENCE DESCRIPTION: SEQ ID NO:51:

GACTATACTA ACAGCATGT

- (2) INFORMATION FOR SEQ ID NO:52:
 - (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 18 base pairs
 - (B) TYPE: nucleic acid
 - (D) TOPOLOGY: unknown
 - (ii) MOLECULE TYPE: DNA (genomic)
 - (xi) SEQUENCE DESCRIPTION: SEQ ID NO:52:

TGTCAGGCAA TGACAAGG

18

- (2) INFORMATION FOR SEQ ID NO:53:
 - (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 26 base pairs
 - (B) TYPE: nucleic acid
 - (D) TOPOLOGY: unknown
 - (ii) MOLECULE TYPE: DNA (genomic)
 - (xi) SEQUENCE DESCRIPTION: SEQ ID NO:53:

AATAGGTCGA GACACTTGTC ACTGGA

- (2) INFORMATION FOR SEQ ID NO:54:
 - (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 29 base pairs
 - (B) TYPE: nucleic acid
 - (D) TOPOLOGY: unknown
 - (ii) MOLECULE TYPE: DNA (genomic)
 - (xi) SEQUENCE DESCRIPTION: SEQ ID NO:54:

CTTGTCACTG GATTTAGATC TCTCAGCTG

- (2) INFORMATION FOR SEQ ID NO:55:
 - (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 30 base pairs
 - (B) TYPE: nucleic acid
 - (D) TOPOLOGY: unknown
 - (ii) MOLECULE TYPE: DNA (genomic)
 - (xi) SEQUENCE DESCRIPTION: SEQ ID NO:55:

GTACACGGCA GGGTCAGGGT TCTGGATATT

(2) INFORMATION FOR SEQ ID NO:56:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 30 base pairs
 - (B) TYPE: nucleic acid
 - (D) TOPOLOGY: unknown
- (ii) MOLECULE TYPE: DNA (genomic)
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:56:

AAGAGAGAC AAAAGGAAAC ATTCTTGAAC

- (2) INFORMATION FOR SEQ ID NO:57:
 - (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 30 base pairs
 - (B) TYPE: nucleic acid
 - (D) TOPOLOGY: unknown
 - (ii) MOLECULE TYPE: DNA (genomic)
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:57:
 GCTGCAAGGC CACATACGAG CAAGGCGTCG
- (2) INFORMATION FOR SEQ ID NO:58:
 - (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 30 base pairs
 - (B) TYPE: nucleic acid
 - (D) TOPOLOGY: unknown
 - (ii) MOLECULE TYPE: DNA (genomic)
 - (xi) SEQUENCE DESCRIPTION: SEQ ID NO:58:

AAAATGAAAG AAAAACCAGA TATTCCTGAG

- (2) INFORMATION FOR SEQ ID NO:59:
 - (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 30 base pairs
 - (B) TYPE: nucleic acid
 - (D) TOPOLOGY: unknown
 - (ii) MOLECULE TYPE: DNA (genomic)
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:59:

CTGAGGCCAC ATATGAGAGT GGATTTGTCA

- (2) INFORMATION FOR SEQ ID NO:60:
 - (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 30 base pairs
 - (B) TYPE: nucleic acid
 - (D) TOPOLOGY: unknown

- (ii) MOLECULE TYPE: DNA (genomic)
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:60:

CAGAGAAACA AAGGAAACTT CCCTGGTCGA

- (2) INFORMATION FOR SEQ ID NO:61:
 - (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 30 base pairs
 - (B) TYPE: nucleic acid
 - (D) TOPOLOGY: unknown
 - (ii) MOLECULE TYPE: DNA (genomic)
- (Xi) SEQUENCE DESCRIPTION: SEQ ID NO:61: GGGTGCGGCA GATGACTCAG GGCTGCCCAA
- (2) INFORMATION FOR SEQ ID NO:62:
 - (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 30 base pairs
 - (B) TYPE: nucleic acid
 - (D) TOPOLOGY: unknown
 - (ii) MOLECULE TYPE: DNA (genomic)
 - (xi) SEQUENCE DESCRIPTION: SEQ ID NO:62:

ATAAATGAAA GTGTGCCAAG TCGCTTCTCA

- (2) INFORMATION FOR SEQ ID NO:63:
 - (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 30 base pairs
 - (B) TYPE: nucleic acid
 - (D) TOPOLOGY: unknown
 - (ii) MOLECULE TYPE: DNA (genomic)
 - (xi) SEQUENCE DESCRIPTION: SEQ ID NO:63:

AACGTTCCGA TAGATGATTC AGGGATGCCC

- (2) INFORMATION FOR SEQ ID NO:64:
 - (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 30 base pairs
 - (B) TYPE: nucleic acid
 - (D) TOPOLOGY: unknown
 - (ii) MOLECULE TYPE: DNA (genomic)
 - (xi) SEQUENCE DESCRIPTION: SEQ ID NO:64:

CATTATAAAT GAAACAGTTC CAAATCGCTT

- (2) INFORMATION FOR SEQ ID NO:65:
 - (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 30 base pairs
 - (B) TYPE: nucleic acid
 - (D) TOPOLOGY: unknown
 - (ii) MOLECULE TYPE: DNA (genomic)
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:65:

CTTATTCAGA AAGCAGAAAT AATCAATGAG

- (2) INFORMATION FOR SEQ ID NO:66:
 - (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 30 base pairs
 - (B) TYPE: nucleic acid
 - (D) TOPOLOGY: unknown
 - (ii) MOLECULE TYPE: DNA (genomic)
 - (xi) SEQUENCE DESCRIPTION: SEQ ID NO:66:

TCCACAGAGA AGGGAGATCT TTCCTCTGAG

- (2) INFORMATION FOR SEQ ID NO:67:
 - (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 30 base pairs
 - (B) TYPE: nucleic acid
 - (D) TOPOLOGY: unknown
 - (ii) MOLECULE TYPE: DNA (genomic)
 - (xi) SEQUENCE DESCRIPTION: SEQ ID NO:67:

GATACTGACA AAGGAGAAGT CTCAGATGGC

- (2) INFORMATION FOR SEQ ID NO:68:
 - (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 30 base pairs
 - (B) TYPE: nucleic acid
 - (D) TOPOLOGY: unknown
 - (ii) MOLECULE TYPE: DNA (genomic)
 - (xi) SEQUENCE DESCRIPTION: SEQ ID NO:68:

GTGACTGATA AGGGAGATGT TCCTGAAGGG

(2) INFORMATION FOR SEQ ID NO:69:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 30 base pairs
 - (B) TYPE: nucleic acid
 - (D) TOPOLOGY: unknown
- (ii) MOLECULE TYPE: DNA (genomic)
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:69:

GATATAAACA AAGGAGAGAT CTCTGATGGA

- (2) INFORMATION FOR SEQ ID NO:70:
 - (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 30 base pairs
 - (B) TYPE: nucleic acid
 - (D) TOPOLOGY: unknown
 - (ii) MOLECULE TYPE: DNA (genomic)
 - (xi) SEQUENCE DESCRIPTION: SEQ ID NO:70:

CATGATAATC TTTATCGACG TGTTATGGGA

- (2) INFORMATION FOR SEQ ID NO:71:
 - (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 30 base pairs
 - (B) TYPE: nucleic acid
 - (D) TOPOLOGY: unknown
 - (ii) MOLECULE TYPE: DNA (genomic)
 - (xi) SEQUENCE DESCRIPTION: SEQ ID NO:71:

TTTCAGAAAG GAGATATAGC TGAAGGGTAC

- (2) INFORMATION FOR SEQ ID NO:72:
 - (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 30 base pairs
 - (B) TYPE: nucleic acid
 - (D) TOPOLOGY: unknown
 - (ii) MOLECULE TYPE: DNA (genomic)
 - (xi) SEQUENCE DESCRIPTION: SEQ ID NO:72:

GATGAGTCAG GAATGCCAAA GGAACGATTT

- (2) INFORMATION FOR SEQ ID NO:73:
 - (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 30 base pairs
 - (B) TYPE: nucleic acid
 - (D) TOPOLOGY: unknown

- (ii) MOLECULE TYPE: DNA (genomic)
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:73: CAAGAAACGG AGATGCACAA GAAGCGATTC
- (2) INFORMATION FOR SEQ ID NO:74:
 - (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 30 base pairs
 - (B) TYPE: nucleic acid
 - (D) TOPOLOGY: unknown
 - (ii) MOLECULE TYPE: DNA (genomic)
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:74:
 ACCGACAGGC TGCAGGCAGG GGCCTCCAGC
- (2) INFORMATION FOR SEQ ID NO:75:
 - (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 30 base pairs
 - (B) TYPE: nucleic acid
 - (D) TOPOLOGY: unknown
 - (ii) MOLECULE TYPE: DNA (genomic)
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:75: CCCTAGCAGG ATCTCATAGA GGATGGTGGC
- (2) INFORMATION FOR SEQ ID NO:76:
 - (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 30 base pairs
 - (B) TYPE: nucleic acid
 - (D) TOPOLOGY: unknown
 - (ii) MOLECULE TYPE: DNA (genomic)
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:76: CCCTAGCAAG ATCTCATAGA GGATGGTGGC
- (2) INFORMATION FOR SEQ ID NO:77:
 - (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 30 base pairs
 - (B) TYPE: nucleic acid
 - (D) TOPOLOGY: unknown
 - (ii) MOLECULE TYPE: DNA (genomic)
 - (xi) SEQUENCE DESCRIPTION: SEQ ID NO:77:

CTCTGCTTCT GATGGCTCAA ACACAGCGAC

- (2) INFORMATION FOR SEQ ID NO:78:
 - (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 30 base pairs
 - (B) TYPE: nucleic acid
 - (D) TOPOLOGY: unknown
 - (ii) MOLECULE TYPE: DNA (genomic)
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:78:
- CTCGGGTGGG AACACCTTGT TCAGGTCCTC
- (2) INFORMATION FOR SEQ ID NO:79:
 - (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 30 base pairs
 - (B) TYPE: nucleic acid
 - (D) TOPOLOGY: unknown
 - (ii) MOLECULE TYPE: DNA (genomic)
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:79:

CTCGGGTGGG AACACGTTTT TCAGGTCCTC

Claims

1. A method of treating rheumatoid arthritis in a mammal comprising:

obtaining a sample of synovium from the mammal;

identifying in said sample T cell receptor variable regions; and

administering to said mammal an effective amount of antibodies to at least one of said T cell receptor variable regions or antigenic fragments thereof.

- 2. The method of claim 1 wherein said mammal is a human.
- 3. The method of claim 1 wherein said sample of synovium is synovial tissue or synovial fluid.
- 4. A method of treating rheumatoid arthritis in a mammal comprising: administering to said mammal an effective amount of antibodies to mammalian T cell receptor variable regions selected from the group consisting of Val7, Val, V β 12, V β 14, V β 17 and V β 7 and antigenic fragments thereof.
 - 5. The method of claim 4 wherein said antibody is specific for at least a portion of one or more peptides having amino acid sequences as set forth in Table 1.
 - 6. The method of claim 4 wherein the mammal is human.
 - 7. A method for immunizing a mammal to prevent the occurrence of rheumatoid arthritis comprising: administering to said mammal mammalian T cell receptor variable regions selected from the group consisting of V α 17, V α 1, V β 12, V β 14, V β 17, V β 7 and antigenic fragments thereof.

WO 93/04695 PCT/US92/07289

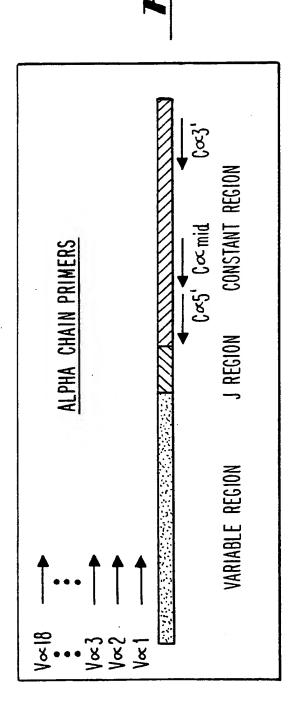
- 95 -

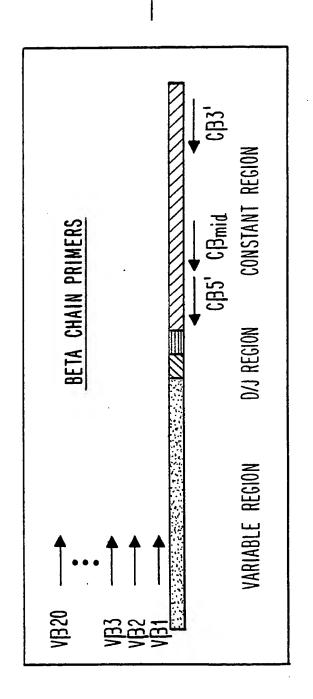
- 8. The method of claim 7 wherein the mammal is a human and the mammalian T cell receptor variable regions are human T cell receptor variable regions.
- 9. The method of claim 7 wherein the mammal is a human and the mammalian T cell receptor variable regions comprise at least a portion of one of the amino acid sequences set forth in Table 1.
- 10. A method for immunizing a mammal to treat rheumatoid arthritis comprising: administering to said mammal mammalian T cell receptor variable regions selected from the group consisting of V α 17, V α 1, V β 12, V β 14, V β 17, V β 7 and antigenic fragments thereof.
- 11. The method of claim 10 wherein the mammal is a human and the mammalian T cell receptor variable regions are human T cell receptor variable regions.
 - 12. The method of claim 10 wherein the mammal is a human and the mammalian T cell receptor variable regions comprise at least a portion of one of the amino acid sequences set forth in Table 1.
 - 13. A kit comprising mammalian T cell receptor variable regions selected from the group consisting of V α 17, V α 1, V β 12, V β 14, V β 17 and V β 7 and antigenic fragments thereof.
 - 14. The kit of claim 13 wherein the mammalian T cell receptor variable regions are human T cell receptor variable regions.
 - 15. The kit of claim 14 wherein the mammalian T cell receptor variable regions comprise at least a portion of one of the amino acid sequences set forth in Table 1.

PCT/US92/07289

- 16. A kit comprising antibodies to mammalian T cell r ceptor variable regions selected from the group consisting of Va17, Va1, V β 12, V β 14, V β 17 and V β 7 and antigenic fragments thereof.
- 17. The kit of claim 16 wherein the mammalian T cell receptor variable regions are human T cell receptor variable regions.
- 18. The kit of claim 17 wherein the variable regions comprise at least a portion of one of the amino acid sequences set forth in Table 1.

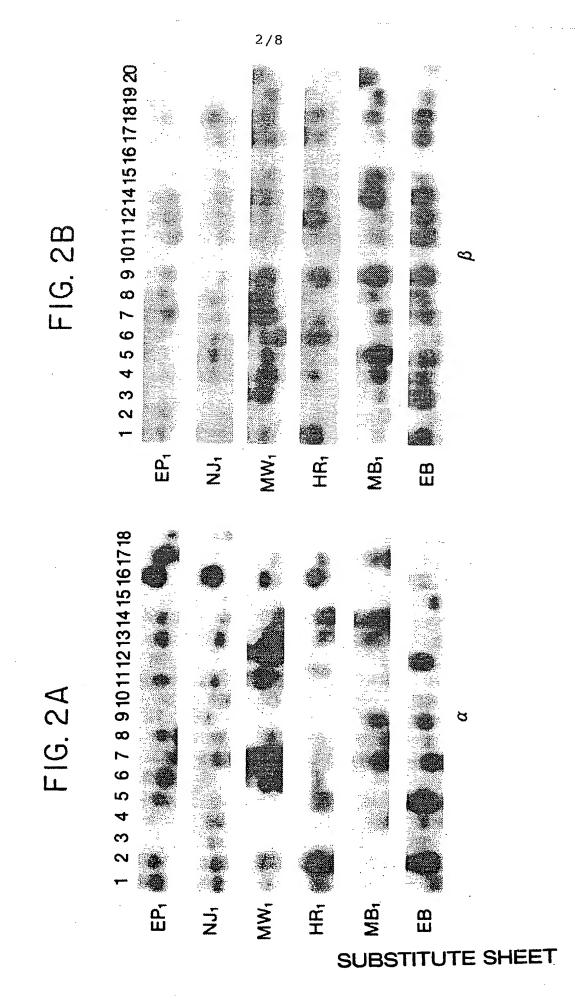
1/8

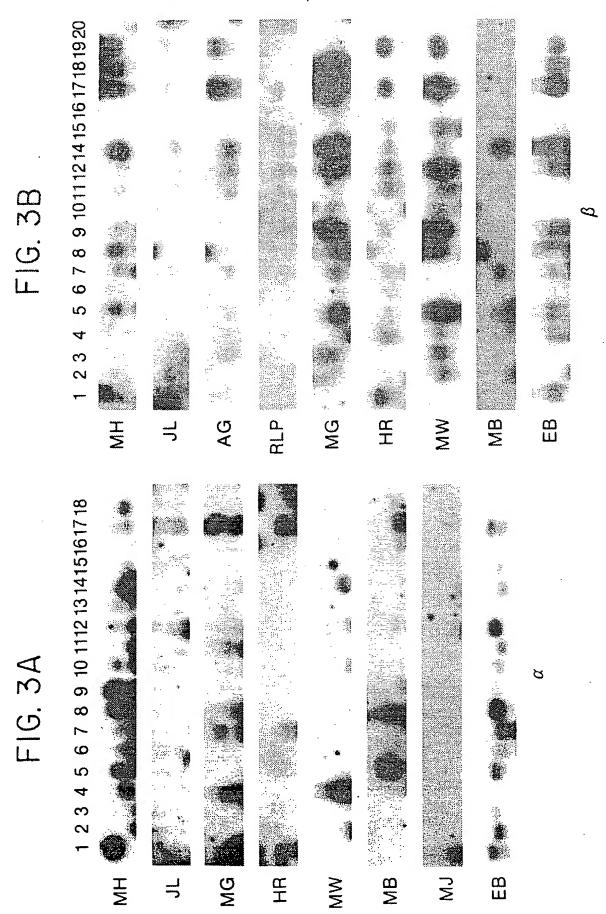




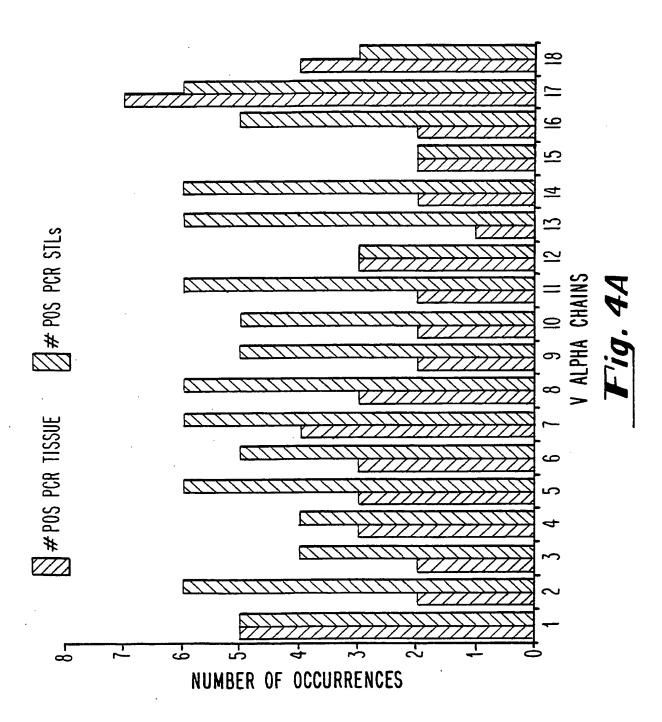
SUBSTITUTE SHEET

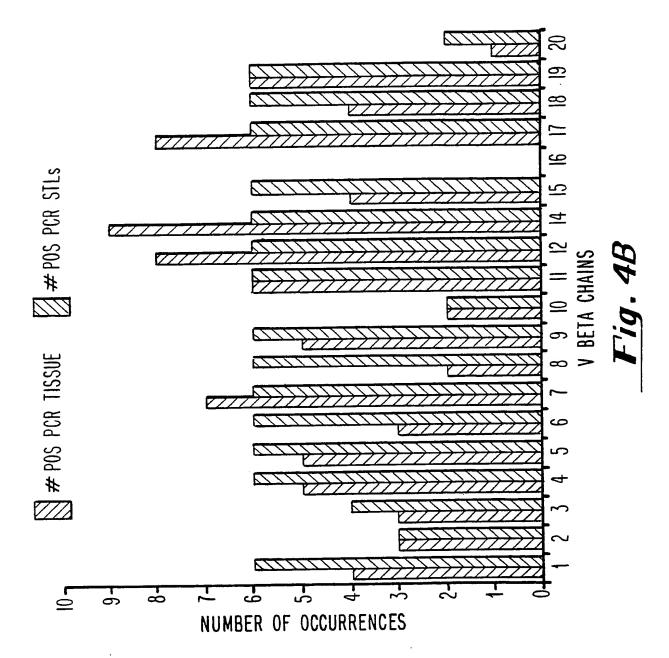
WO 93/04695 PCT/US92/07289





SUBSTITUTE SHEET





6/8

FIGURE 5

Vαl	CTGAGGTGCAACTACTCA
Va2	GTGTTCCCAGAGGGAGCCATTGCC
Vα3	GGTGAACAGTCAACAGGGAGA
Va4	ACAAGCATTACTGTACTCCTA
Va5	GGCCCTGAACATTCAGGA
Va6	GTCACTTTCTAGCCTGCTGA
Vα7	AGGAGCCATTGGTCCAGATAAA
Vα7	GGAGAATGTGGAGCAGCATC
Vα9	ATCTCAGTGCTTGTGATAATA
Val0	ACCCAGCTGGTGGAGCAGAGCCCT
Vall	AGAAAGCAAGGACCAAGTGTT
	CAGAAGGTAACTCAAGCGCAGACT
Vα12	GCTTATGAGAACACTGCGT
Vα13	GCAGCTTCCCTTCCAGCAAT
Vα14	AGAACCTGACTGCCCAGGAA
Vα15	CATCTCCATGGACTCATATGA
Vα16	GACTATACTAACAGCATGT
Vα17	TGTCAGGCAATGACAAGG
Vα18	AATAGGTCGAGACACTTGTCACTGGA
*Cα3 !	CTTGTCACTGGATTTAGATCTCTCAGCTG
*Camid	GTACACGGCAGGGTCAGGGTTCTGGATATT
*Cα5'	GTACACGGCAGGGTCAGGGTTCTGGATATT
V β 1	AAGAGAGAGCAAAAGGAAACATTCTTGAAC
Vβ2	GCTGCAAGGCCACATACGAGCAAGGCGTCG
Vβ3	AAAATGAAAGAAAAAGGAGATATTCCTGAG
Vβ4	CTGAGGCCACATATGAGAGTGGATTTGTCA
Vβ5	CAGAGAAACAAAGGAAACTTCCCTGGTCGA
Vβ6	GGGTGCGGCAGATGACTCAGGGCTGCCCAA
Vβ7	ATAAATGAAAGTGTGCCAAGTCGCTTCTCA
Vβ8	AACGTTCCGATAGATGATTCAGGGATGCCC
Vβ9	CATTATAAATGAAACAGTTCCAAATCGCTT
V <i>β</i> 10	CTTATTCAGAAAGCAGAAATAATCAATGAG
Vβ11	TCCACAGAGAAGGGAGATCTTTCCTCTGAG
Vβ12	GATACTGACAAAGGAGAAGTCTCAGATGGC
Vβ14	GTGACTGATAAGGGAGATGTTCCTGAAGGG
Vβ15	GATATAAACAAAGGAGAGATCTCTGATGGA
Vβ16	CATGATAATCTTTATCGACGTGTTATGGGA
Vβ17	TTTCAGAAAGGAGATATAGCTGAAGGGTAC
Vβ18	GATGAGTCAGGAATGCCAAAGGAACGATTT
Vβ19	CAAGAAACGGAGATGCACAAGAAGCGATTC
Vβ20	ACCGACAGGCTGCAGGCAGGGGCCTCCAGC
*Cβ ₁ 3'	CCCTAGCAGGATCTCATAGAGGATGGTGGC
*Cβ ₂ 3 '	CCCTAGCAAGATCTCATAGAGGATGGTGGC
*Cβmid	CTCTGCTTCTGATGGCTCAAACACAGCGAC
*Cβ15'	CTCGGGTGGGAACACCTTGTTCAGGTCCTC
*Cβ ₁ 5 '	CTCGGGTGGGAACACGTTTTTCAGGTCCTC
~Cp ₂ S	010001000111011001111111111111111111111

S 8		+		ļ							
S 6	+	+	+			+	+			+	9
<u>E</u> 80	+	+			+			:		+	4
₩ HB	+	+	+	+	+	+	+			+	*8
VВ 15		+				+	+			+	4
C 본	+	+	+	+	+	+	+	+		+	*6
당 2	+	+	+	+	+	+	+			+	*8
웅=	+	+	+			+	+			+	9
뜻으					+	رن	+		c.		2
E/ 6	+				+	+	+			+	5
8 8	+	c·	~·		ر.	<i>د</i> .	<i>ر</i> ۔	<i>ر</i> .	į	+	2
VB ~	+	+	+		+	+		+		+	١
γ 9	+					+			-	+	3
V3	+		+			+	+			+	5
V3	+				+	+	+			+	5
₩ 3			+		+		+				3
₽~	+	<i>ر</i> -،					+			+	3
Vβ +	+	٠.	+			+				+	4
Pt	HW.	7	AG	RLP	MG	HR	MM	₩	ω	83	+ #



× 8 8	+		+	-						+	4
Λα 7	+	+	+		+	+		+		+	7*
γ _α				+						+	2
γα 15	+									+	2
γ 4	٠.						+			+	2
۲ ا	+										1
ج ا	+	+							+	+	3
ج ا					į					+	2
<u>ه</u> ح	+									+	2
×وص	+.									+	7
× ∞	+							+		+	3
۸۶	+				+	+				+	4
و لا	+	2						+		+	3
\ S S	+							-		+	3
> 4	+				+		+				3
۸ م	+									+	2
کم ح	+									+	2
×-	+				+	+			+	+	5
0	풀	7	AG	RLP	WG	Æ	Mω	MB	£	83	+#

Fig. 7

		PCT/US92/072	289			
IPC(5) US CL	ASSIFICATION OF SUBJECT MATTER :A61K 39/00, 39/395; C07K 7/10, 15/06, 15/28; G :Please See Extra Sheet. to International Patent Classification (IPC) or to both					
	LDS SEARCHED					
	locumentation searched (classification system follower	ed by classification symbols)				
U.S. :	530/324, 350,388.22, 388.75, 388.85, 389.1, 389.6	; 424/85.8, 88; 435/7.24				
Documentat	tion searched other than minimum documentation to th	e extent that such documents are included	in the fields searched			
	data base consulted during the international search (n LINE, BIOSIS	ame of data base and, where practicable	, search terms used)			
C. DOC	CUMENTS CONSIDERED TO BE RELEVANT					
Category*	Citation of document, with indication, where a	ppropriate, of the relevant passages	Relevant to claim No.			
<i>t</i> .	US, A, 4,886,743 (Hood et al.) 12 December 198	9, Abstract and claims 54-55.	1-6, 16-18			
,	WO, A, 90/11294 (HOWELL ET AL) 04 October	1990, page 16 and claims 20-24.	1-18			
	Eur. J. Immunol., Volume 20, issued 1990, S prevention of adjuvant arthritis in rats by a monocreceptor ", pages 2805-2808, especially page 2805	clonal antibody to the alpha/beta T cell	1-6, 16-18			
			·			
X Furth	er documents are listed in the continuation of Box C	See patent family annex.				
A" doc	ecial categories of cited documents: cument defining the general state of the art which is not considered	"T" later document published after the inte date and not in conflict with the applic principle or theory underlying the inv	ation but cited to understand the			
E" earl	be part of particular relevance lier document published on or after the international filing date	"X" document of particular relevance; the considered novel or cannot be considered.				
cite	rument which may throw doubts on priority claim(s) or which is at to establish the publication date of another citation or other cital reason (as specified)	"Y" document of particular relevance; the considered to involve an inventive				
me		combined with one or more other such being obvious to a person skilled in th	a documents, such combination se art			
the	priority date claimed	*&* document member of the same patent				
	actual completion of the international search MBER 1992	Date f mailing of the international search report 24 NOV 1992				
lame and m Commission Box PCT	nailing address of the ISA/ ner of Patents and Trademarks	Authorized officer CHDISTINA CHAN				

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.		
(Brit. J. Rheumatol., issued 1991, G. Kingsley, "Monoclonal antibody treatment of rheumatoid arthritis", pages 33-35, especially page 34.	1-6, 16-18		
(Proc. Natl. Acad. Sci. USA, Volume 85, issued November 1988, K. Sakai et al., "Involvement of distinct murine T-cell receptors in the autoimmune encephalitogenic response to nested epitopes of myelin basic protein ", pages 8608-8612, especially page 8612.	1-18		
r	Clin. Exp. Immunol., Volume 49, issued 1982, O. Duke et al., "An immunohistological analysis of lymphocyte subpopulations and their microenvironment in the synovial membranes of patients with rheumatoid arthritis using monoclonal antibodies ", pages 22-30, especially page 22.	1-18		
	Science, Volume 253, issued 19 July 1991, X. Paliard et al., " Evidence for the effects of a superantigen in rheumatoid arthritis ", pages 325-329, especially page 325.	1-18		
,	Proc. Natl. Acad. Sci USA, Volume 86, issued November 1989, Y. Choi et al., "Interaction of Staphylococcus aureus toxin "superantigens with human T cells , pages 8941-8945, especially page 8945.	1-18		
•	Nature, Volume 341, issued 12 October 1989, A. Vandenbark et al., "Immunization with a synthetic T-cell receptor V-region peptide protects against experimental autoimmune encephalomyelitis", pages 541-544, especially page 541.	7-15		

nternational application No. PCT/US92/07289

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)	
This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:	
1. Claims Nos.: because they relate to subject matter not required to be searched by this Authority, namely:	
Claims Nos.: because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:	
3. Claims Nos.: because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).	
Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)	
This International Searching Authority found multiple inventions in this international application, as follows: 1. Claims 1-6 and 16-18, drawn to a method and a kit involving antibodies, classified in Class 424 Subclass 85.8. II. Claims 7-15, drawn to a method and a kit involving T cell receptor variable regions, classified in Class 424 Subclass The inventions as grouped are distinct, each from the other, because they represent different inventive endeavors. The method and the kit in Group I would not suggest the method and the kit in Group II. They are unrelated in operation and one does require the other for ultimate use and the specification does not disclose a dependent relationship among them.	100
1. X As all required additional search fees were timely paid by the applicant, this international search report covers all searchab claims. (Telephone Practice)	le
2. As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.	ıt
3. As only some of the required additional search fees were timely paid by the applicant, this international search report cover only those claims for which fees were paid, specifically claims Nos.:	rs
No required additional search fees were timely paid by the applicant. Consequently, this international search report restricted to the invention frist mentioned in the claims; it is covered by claims Nos.:	s
Remark on Protest The additional search fees were accompanied by the applicant's protest. No protest accompanied the payment of additional search fees	

A. CLASSIFICATION OF SUBJECT MATTER: US CL :

530/324, 350,388.22, 388.75, 388.85, 389.1, 389.6; 424/85.8, 88; 435/7.24

.